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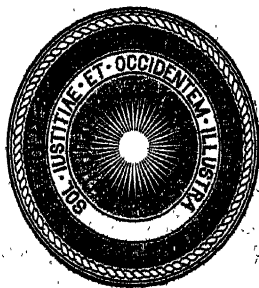


# SOIL SCIENCE

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# SOIL SCIENCE

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# SOIL SCIENCE

A MONTHLY JOURNAL DEVOTED TO PROBLEMS  
IN SOIL PHYSICS, SOIL CHEMISTRY AND  
SOIL BIOLOGY

JACOB G. LIPMAN

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## ERRATA

Vol. 5, No. 5, page 397, table 2, last two columns of lower half of table should read:

	AVERAGE HEIGHT, OF PLANTS	NUMBER OF HEADS
1-6 inches { 1.....	27	33
{ 2.....	28	35
Average.....	27.5	34
Third foot { 1.....	18	8
{ 2.....	17	6
Average.....	17.5	7
Fifth foot { 1.....	14.5	4
{ 2.....	14	8
Average.....	14.2	6

# A COMPARATIVE STUDY OF SALT REQUIREMENTS FOR YOUNG AND FOR MATURE BUCKWHEAT PLANTS IN SAND CULTURES

JOHN W. SHIVE

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Received for publication June 10, 1918

In a recent paper (9) presenting the results of a comparative study of salt requirements for buckwheat plants grown in solution cultures during two different periods of their development, it was announced that similar experiments were carried out with these plants in sand cultures corresponding to the solution cultures. The results of this experimental work with buckwheat in sand cultures furnishes the subject matter of the present paper, which also compares these results with those obtained from the corresponding solution cultures of the earlier work.<sup>1</sup>

As previously stated, the object of these investigations was to determine the salt requirements demanded for approximately optimum growth of buckwheat plants during the early stages of their development while the vegetative processes are extremely active, and to compare these with the relative salt proportions best adapted to the development of these plants during the reproductive stages and during the period of seed formation. The entire active life period of the plants was thus divided into two partial periods extending over nearly equal intervals of time, these partial periods representing distinct physiological phases of development. The first of these covered the period between the germination of the seeds and the beginning of the flowering stage, while the second extended from the close of the first period to the maturity of the seeds.

## EXPERIMENTAL PROCEDURE AND METHODS

### *1. The culture solutions*

Throughout these tests an optimal series of 3-salt solutions (7) was employed in sand cultures. This series was composed of 36 different solutions including all the possible proportions of the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulfate, when the partial concentrations of each of the components were made to vary by equal increments of one-tenth

<sup>1</sup> A preliminary report giving some of the principal results of these studies has already appeared: Shive, J. W., and Martin, W. M., Ref. No. 8.

of the total osmotic concentration. Each solution had an initial total concentration value of approximately 1.75 atmospheres. Previous experiments have shown that this concentration is well within the range required for optimum growth of buckwheat. The partial osmotic concentration values, as well as the volume-molecular partial concentrations of each salt in these solutions, have been calculated according to the method employed by Tottingham (10, p. 177-182 and 192). A table giving the formulas of the solutions of this series has already appeared in several publications (5, 7) and need not be repeated here. The methods used in preparing the stock solutions, and the necessary manipulations with reference to the making up of the nutrient solutions were practically the same as were those previously described (7).

## 2. The sand cultures with renewal of solutions

The substratum employed with the cultures of these tests consisted of white quartz sand which had previously been thoroughly washed with tap water followed with distilled water. For the first washing the sand was placed in a granite-ware tub and a stream of water from a hose was then directed into the sand, allowing the water to overflow the sides of the tub while the sand was constantly being agitated. This process was continued until the water overflowing the tub was clear of all sediment. The surplus tap water was then decanted, after which the sand was washed twice with distilled water by pouring the water on the sand in the tub and stirring the sand in this water. After the final washing with distilled water the sand was spread on large sheets of paper until air-dry. This sand had a water-holding capacity of 24.9 per cent (average of six determinations) on the dry-weight basis, determined according to the method of Hilgard (2), which employs sheet metal pans with lateral walls 1 cm. high, the bottoms being perforated. The percentages of different-sized particles of sand, obtained by a mechanical separation, are given in table 1.

TABLE 1

*Mechanical analysis of sand giving the percentages of the different-sized particles*

	<i>per cent</i>
Gravel (more than 2.0 mm.).....	0.58
Fine gravel (2.0 mm. to 1.0 mm.).....	5.67
Coarse sand (1.0 mm. to 0.5 mm.).....	30.83
Medium sand (0.5 mm. to 0.317 mm.).....	53.89
Fine sand (0.317 mm. to 0.254 mm.).....	7.36
Very fine sand (less than 0.254 mm.).....	1.67

The culture vessels consisted of glazed earthenware pots, each with a capacity of about 2 liters; 2.5 kgm. of sand were used for each culture. To provide for the removal of the solutions from the sand cultures, a method similar to that devised by McCall (4) was here adopted. A glass tube with an inside diameter of 4 mm. was placed vertically against the wall of the pot, extending through the sand to the bottom of the pot. The tube was screened at its

lower end by means of a plug of glass wool which prevented the escape of any grains of sand when suction was applied to the top of the tube. To prevent the plug from being drawn through the tube when suction was applied for the removal of the solution, a tuft of the glass wool attached to the plug was allowed to extend 1.5 cm. to 2.0 cm. beyond the end of the glass tube. The sand resting upon this tuft held the plug firmly in place. After the sand (2500 gm.) had been weighed into the pot, a paraffined paper funnel was placed in the inverted position at the center of the sand surface. The lower end of the funnel was buried in the sand to the depth of about 1 cm. These funnels, which were about 6 cm. in length with a diameter of 2 cm. at one end and about 4 cm. at the other, were made of heavy paper and were then thoroughly impregnated with melted paraffine to render them impervious to moisture. To prepare the dry sand in each pot for the planting of the seedlings, the nutrient solution was poured into the sand through the funnel until the sand was nearly saturated. The sand culture was then ready to receive the seedlings.

The "Japanese" variety of buckwheat was employed. The seed used was from the same lot as was used in the earlier work with solution cultures corresponding to the sand cultures of the present study. The seeds were germinated and the seedlings grown on a germinating net in the manner described in a previous publication (7). When the seedlings were about 5 cm. tall, those selected for uniformity were carefully transferred to the pots of sand which had previously been prepared in such a way as to provide for the renewal of the solutions in the sand at regular intervals during the growth period. Five seedlings were transplanted to each pot, after which a sufficient amount of the solution was added through the funnel to flood the culture until the free solution stood above the sand to the depth of 1 cm. or more. This served to fix the roots of the seedlings in place and to smooth the surface of the sand. With this initial application, 750 cc. of solution were added to the sand of each culture. Suction was then applied to the top of the glass tube (provided for the removal of the solution) by means of an aspirating pump and the withdrawal of the solution was continued until the sand was reduced to the desired moisture content, which throughout these tests was maintained at approximately 15 per cent of the dry weight of the sand, or 60 per cent of its water-holding capacity. Two more portions of the solution (250 cc. to each portion) were then passed through the sand, the moisture content being reduced each time to the desired 60 per cent of its moisture-holding capacity. The culture was then sealed. This was done by pouring over the surface of the sand a thin layer of melted Briggs and Shantz (1) wax, thus completely covering the sand between the walls of the pot and the funnel. The sealing of the cultures was rendered necessary in order to prevent evaporation and the consequent disturbing factor of salt precipitation at the surface of the sand, and in order also to control the concentration of the nutrient solution and to make possible the measurement of water loss by transpiration.

At the end of each three or four day period, each pot was weighed and a sufficient amount of distilled water was added through the funnel to restore the entire system to its original weight, after which a fresh nutrient solution was added (250 cc. to each culture) while at the same time an equal quantity was withdrawn through the glass tube provided for the purpose.

The transpirational water loss from the plants, during a given interval of time, is supplied, of course, through the absorption by the roots of an approximately equal quantity from the solution in the medium in which the plants are rooted. This process results in a gradual increase in the concentration of the solution. In order to prevent any excessive variations in the concentration of the nutrient solutions, due to the absorption by the roots, the cultures were weighed at 2-day intervals during the early growth stages and daily during the later stages of growth. At each weighing a sufficient quantity of distilled water was added to each culture to restore that which had been absorbed by the roots during the interval in question. The amount of water added to each culture at any weighing was approximately equal, of course, to the amount lost by transpiration during the interval directly preceding the weighing. A record was kept of the amounts of water added from time to time. The total amount of water lost during the entire growth period was obtained by summing the losses for the partial periods between each two successive weighings.

### *3. Early period of development*

In order to determine the best proportions of the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ , for the growth of buckwheat tops and of roots in sand cultures during the early developmental period, between the germination of the seed and the flowering stage, 36 sand cultures were prepared, as above described, with the 36 different solutions of the 3-salt series employed in these tests. All the seedlings used were selected for uniformity of size and vigor. These were carefully removed from the germination net, one at a time, when about 4 cm. tall, and were transplanted to the sand cultures previously prepared, 5 seedlings to each culture. One culture was prepared also with Knop's solution and another with Tottingham's best solution for wheat tops, each with a total osmotic concentration value (1.75 atmospheres) equal to that of the solutions of the 3-salt series. These cultures were added to the series for comparison.

This series of sand cultures was now continued with renewal of solutions every three or four days, until the plants began to bloom. This required a time period of 25 days after the seedlings had been transferred to the sand cultures. The series was then repeated.

At the end of the growth period the wax seal was removed from the cultures and the tops of the plants were severed from the roots just at the surface of the sand. The tops were then dried to constant weight at a temperature of

about 103°C. and the dry weights obtained. The method employed in harvesting the roots was essentially the same as that adopted by McCall (5). In order to wash the roots as free from sand as possible, the contents of each culture pot, after the tops of the plants had been removed, were transferred to a sieve with meshes sufficiently large to allow all the sand grains to pass through. The sand was then washed through the sieve by means of a gentle stream of water, leaving the roots on the sieve. The roots, together with some adhering sand grains, were dried to constant weight in the same manner as were the tops. The dried roots with the adhering sand were then weighed, after which the roots were ignited in crucibles of fused silica until all the organic matter had been destroyed. The loss in weight due to the ignition process was taken to represent the approximate dry weight of the roots, assuming, of course, that the adhering sand suffered no loss in weight in the ignition process. The small amount of ash resulting from the ignition of the roots was considered negligible, since, as McCall pointed out, the relative weights would be affected only by the differences between the weights of ash from the various individual cultures.

Throughout these experiments daily records were kept of the temperature and moisture conditions in the greenhouse where the cultures were conducted. Maximum and minimum temperature readings were obtained from thermometers protected from direct sunlight, and the evaporating power of the air was measured by means of the daily rates of water loss from standardized, spherical, porous-cup atmometers. The readings obtained from these instruments were corrected to the Livingston (3) standard spherical cup by multiplying the actual readings by the coefficient of correction of the cups used.

The first of the two series conducted during the early developmental period extended from April 12 to May 7, 1917. During the period of this series the highest temperature recorded was 33°C. (on April 23), and the lowest was 7°C. (on April 18). The water loss from the porous cup atmometer gave a daily mean of 9.8 cc., a maximum daily rate of 20.9 cc. (on April 18), a minimum daily rate of 2.9 cc. (on April 27), and a total loss from the instrument of 244.6 cc. for the entire period. The second series, which was just like the first, was carried out between May 24 and June 18, 1917. During this time the maximum temperature reached was 35°C. (on June 11), and the minimum was 9°C. (on May 26). The evaporation rate from the atmometer gave a daily mean of 12.6 cc., a maximum daily rate of 21.6 cc. (on June 4), a minimum daily rate of 2.0 cc. (on May 29), and a total loss of 313.6 cc. for the entire period. For the sake of convenience in presenting the data, this double series will be designated series A throughout.

#### *4. Late period of development*

The tests dealing with the questions of salt requirements for the buckwheat during the later growth period were begun with plants which had reached the stage of development attained by those harvested at the end of the early

period of growth, when the plants had just reached the flowering stage. In order to obtain a sufficient number of plants at this stage of development, which were all nearly alike in size and vigor, the following procedure was adopted:

A larger number of sand cultures than that required for the series was prepared. Carefully selected seedlings were transplanted to these cultures, five seedlings to each culture, in the manner above described. Each of these sand cultures was provided with the solution<sup>2</sup> of the optimal 3-salt series producing the highest yield of buckwheat tops and roots during the first four weeks after germination. These cultures were continued to the flowering stage, with renewal of solutions every third or fourth day, covering an early growth period of 25 days after the seedlings had been transferred to the sand cultures. During this time all the plants were grown in the same nutritive medium and under approximately similar conditions of temperature, light, and moisture. This procedure gave very uniform plants.

At the end of the early 25-day growth period, 36 cultures were selected from the larger number at hand. The solutions in these and cultures were now replaced by the 36 different solutions of the optimal 3-salt series. This was accomplished by passing through the sand of each culture (after first adding sufficient distilled water to bring the entire system back to its original weight) a triple portion (750 cc.) of the new solution, thus flushing out the old solution and replacing it with the new. One culture with Knop's solution and one with Totttingham's best solution for wheat tops were also included in the series for comparison. These were treated in the same manner as were the other cultures of the series. The series was now continued with renewal of solutions every third or fourth day as before, until practically all the seeds were ripe. This second, or late, developmental period extended over a time interval of 30 days. The entire active growth period of the plants, after the seedlings had been transferred to the sand cultures, extended over an interval of 55 days.

At the end of the active growth period the plants were harvested and the dry weight yields obtained in the same manner as were those of series A, representing the early developmental period. The yields of tops, roots and seeds were obtained separately.

During the active growth period, extending from April 25 to June 19, the maximum temperature experienced by the cultures was 35°C. (on June 11), and the minimum was 9°C. (on May 26). The water loss from the atmometer, indicating the evaporating power of the air, gave a daily mean of 12.4 cc., a maximum and a minimum daily rate of 23.2 cc. and 2.0 cc. on June 5 and May 29, respectively, and a total loss from the instrument of 680.2 cc. for the entire time period. The series was repeated between July 2 and August 27. During this time a maximum temperature of 38°C.

<sup>2</sup> This solution contained the three salts in the following volume-molecular partial concentrations:  $\text{KH}_2\text{PO}_4$ , 0.144 m.;  $\text{Ca}(\text{NO}_3)_2$ , 0.0052 m.; and  $\text{MgSO}_4$ , 0.0200 m.

was reached on August 1, and a minimum of 14° C. on July 12. The rate of evaporation from the atmometer gave a daily mean of 17.1 cc., a maximum and a minimum daily rate of 35.1 cc. and 3.3 cc. on August 26 and July 12, respectively, and a total loss of 938.9 cc. for the entire time period. This double series will be designated series B, throughout this study.

#### EXPERIMENTAL RESULTS

As previously stated, a comparative study of the growth of buckwheat plants in water cultures, corresponding to the present study with sand cultures, has already been carried out. The methods of presenting the results obtained with the two double series of cultures here considered will, therefore, follow the same general outline as that employed in the earlier work (9).

The behavior of the *young* buckwheat plants (series A, early developmental period) in the different sand cultures provided with the solutions of the optimal 3-salt series, will be compared with the behavior of the *older* plants (series B, late period of development) grown in sand cultures provided with solutions of the same series. The comparison will be made by means of three kinds of direct quantitative measurements: (1) dry weights of tops, (2) dry weights of roots, and (3) total transpirational water loss from the plants during the entire growth periods. Further comparisons will be made of two other quantitative criteria derived from the transpiration values considered in connection with the dry weights of tops and of roots. These are (1) water requirements of tops and (2) water requirements of roots. These values represent the transpirational water loss for each single gram of dry plant substance produced. The dry weights of seeds will also be considered in connection with the data of series B.

#### *I. Dry-weight yields*

##### A. Presentation of data

Since the tops and roots of series A, and the tops, roots, and seeds of series B were weighed separately, two sets of dry-weight measurements are available for the former series and three for the latter. The dry-weight values of tops and of roots of series A are presented in table 2, and in a similar manner those of series B are given in table 3. In the first column of each table are given the culture numbers referring to the positions which the cultures occupy on the triangular diagram<sup>3</sup> graphically representing the variations in the salt proportions of the culture solutions of the series here employed in sand cultures.

Since, as above stated, each of the two series was repeated, each dry weight datum, as given in the tables, represents the average yield obtained from two corresponding cultures. The tables give the average absolute dry weights, in grams, and also the relative values of these in terms of the corresponding value

<sup>3</sup> For the description of this diagram see Shive (7) and McCall (5).



TABLE 2

*Average dry weights of tops and roots of buckwheat grown to the flowering stage in sand cultures supplied with three-salt solutions, all having a total osmotic concentration value of 1.75 atmospheres, but differing from each other in the proportions of the constituent salts*

CULTURE NUMBER	AVERAGE DRY-WEIGHT YIELDS			
	Tops (5 plants)		Roots (5 plants)	
	Absolute	Relative to $R_1C_1$ as unity	Absolute	Relative to $R_1C_1$ as unity
	gm.		gm.	
$R_1C_1$	1.875	1.00	0.276	1.00
$C_2$	2.850	1.52	0.292	1.06
$C_3$	2.858	1.52	0.250	0.91
$C_4$	3.145	1.68	0.334	1.21
$C_5$	3.072	1.64	0.328	1.19
$C_6$	3.186	1.70	0.333	1.21
$C_7$	3.642	1.94	0.363	1.31
$C_8$	2.860	1.52	0.294	1.06
$R_2C_1$	2.023	1.08	0.224	0.81
$C_2$	3.492	1.86	0.382	1.38
$C_3$	3.648	1.95	0.376	1.36
$C_4$	3.423	1.82	0.316	1.15
$C_5$	3.566	1.90	0.384	1.39
$C_6$	3.854	2.05	0.409	1.48
$C_7$	2.803	1.49	0.318	1.15
$R_3C_1$	2.560	1.36	0.279	1.01
$C_2$	3.825	2.04	0.312	1.13
$C_3$	3.542	1.89	0.427	1.55
$C_4$	3.456	1.84	0.406	1.47
$C_5$	3.508	1.87	0.391	1.42
$C_6$	3.164	1.69	0.317	1.15
$R_4C_1$	2.695	1.44	0.339	1.23
$C_2$	4.457	2.38	0.373	1.35
$C_3$	4.141	2.20	0.388	1.41
$C_4$	3.748	2.00	0.402	1.46
$C_5$	3.746	2.00	0.398	1.44
$R_5C_1$	2.228	1.19	0.235	0.85
$C_2$	2.971	1.58	0.287	1.04
$C_3$	3.888	2.08	0.319	1.15
$C_4$	3.707	1.98	0.456	1.65
$R_6C_1$	2.382	1.27	0.298	1.08
$C_2$	3.397	1.81	0.399	1.44
$C_3$	3.472	1.85	0.358	1.30
$R_7C_1$	2.419	1.29	0.291	1.05
$C_2$	2.996	1.60	0.316	1.14
$R_8C_1$	2.515	1.34	0.200	0.73
K*	3.187	1.70	0.319	1.15
T*	3.577	1.91	0.392	1.42

\* In this table and in subsequent tables K and T represent cultures prepared with Knop's solution and with Tottingham's best solution, respectively. The data obtained from these cultures are introduced for comparison.

TABLE 3

*Average dry weights of tops, roots, and seeds of buckwheat grown from the flowering stage to maturity in sand cultures supplied with three-salt solutions, all having a total osmotic concentration value of 1.75 atmospheres but differing from each other in the proportions of the constituent salts*

CULTURE NUMBER	AVERAGE DRY-WEIGHT YIELDS						RATIO OF TOPS TO SEEDS
	Tops (5 plants)		Roots (5 plants)		Seeds (5 plants)		
	Absolute	Relative to R <sub>1</sub> C <sub>1</sub> as unity	Absolute	Relative to R <sub>1</sub> C <sub>1</sub> as unity	Absolute	Relative to R <sub>1</sub> C <sub>1</sub> as unity	
	gm.		gm.		gm.		
R <sub>1</sub> C <sub>1</sub>	5.345	1.00	0.690	1.00	2.980	1.00	1.79
C <sub>2</sub>	6.346	1.17	0.783	1.13	3.332	1.12	1.91
C <sub>3</sub>	7.696	1.44	1.003	1.45	4.328	1.45	1.78
C <sub>4</sub>	8.825	1.65	0.905	1.31	4.694	1.57	1.88
C <sub>5</sub>	8.957	1.68	0.935	1.36	4.306	1.45	2.08
C <sub>6</sub>	7.523	1.40	0.723	1.05	3.696	1.24	2.03
C <sub>7</sub>	8.168	1.53	0.828	1.20	2.804	0.94	2.92
C <sub>8</sub>	9.287	1.72	0.812	1.18	2.681	0.90	3.46
R <sub>2</sub> C <sub>1</sub>	5.599	1.05	0.680	0.99	2.246	0.76	2.49
C <sub>2</sub>	6.678	1.25	0.737	1.07	2.137	0.72	3.12
C <sub>3</sub>	8.863	1.66	0.985	1.43	3.176	1.07	2.78
C <sub>4</sub>	8.889	1.66	1.029	1.49	3.895	1.31	2.28
C <sub>5</sub>	9.364	1.75	1.142	1.65	4.230	1.42	2.21
C <sub>6</sub>	9.599	1.80	1.132	1.64	3.058	1.03	3.14
C <sub>7</sub>	8.135	1.52	0.926	1.34	3.260	1.09	2.51
R <sub>3</sub> C <sub>1</sub>	7.911	1.48	0.691	1.00	2.120	0.71	3.73
C <sub>2</sub>	8.492	1.59	0.651	0.94	2.647	0.89	3.20
C <sub>3</sub>	8.544	1.60	1.157	1.68	3.174	1.06	2.69
C <sub>4</sub>	10.298	1.93	1.114	1.63	4.249	1.43	2.42
C <sub>5</sub>	12.314	2.30	1.300	1.88	5.001	1.68	2.46
C <sub>6</sub>	9.501	1.78	1.117	1.62	4.304	1.44	2.21
R <sub>4</sub> C <sub>1</sub>	6.783	1.27	0.768	1.11	2.325	0.78	2.92
C <sub>2</sub>	8.316	1.56	1.063	1.54	3.752	1.26	2.22
C <sub>3</sub>	8.726	1.64	0.886	1.28	4.190	1.41	2.08
C <sub>4</sub>	9.853	1.84	1.215	1.76	3.238	1.09	3.04
C <sub>5</sub>	6.831	1.28	0.805	1.17	3.122	1.05	2.19
R <sub>5</sub> C <sub>1</sub>	5.528	1.03	0.795	1.15	3.651	1.22	1.51
C <sub>2</sub>	8.772	1.64	1.017	1.47	2.204	0.74	3.98
C <sub>3</sub>	8.943	1.67	1.087	1.58	3.594	1.20	2.49
C <sub>4</sub>	9.160	1.71	0.873	1.27	2.657	0.89	3.44
R <sub>6</sub> C <sub>1</sub>	6.000	1.12	0.788	1.14	2.754	0.92	2.18
C <sub>2</sub>	8.255	1.55	1.085	1.57	3.761	1.26	2.20
C <sub>3</sub>	7.973	1.49	0.535	0.78	3.257	1.09	2.44
R <sub>7</sub> C <sub>1</sub>	6.231	1.16	0.802	1.16	3.105	1.04	2.00
C <sub>2</sub>	7.023	1.31	0.682	0.97	3.250	1.09	2.16
R <sub>8</sub> C <sub>1</sub>	5.897	1.10	0.677	0.98	2.830	0.95	2.08
K	8.708	1.63	1.070	1.55	4.046	1.36	2.15
T	8.737	1.64	0.907	1.31	3.456	1.16	2.53

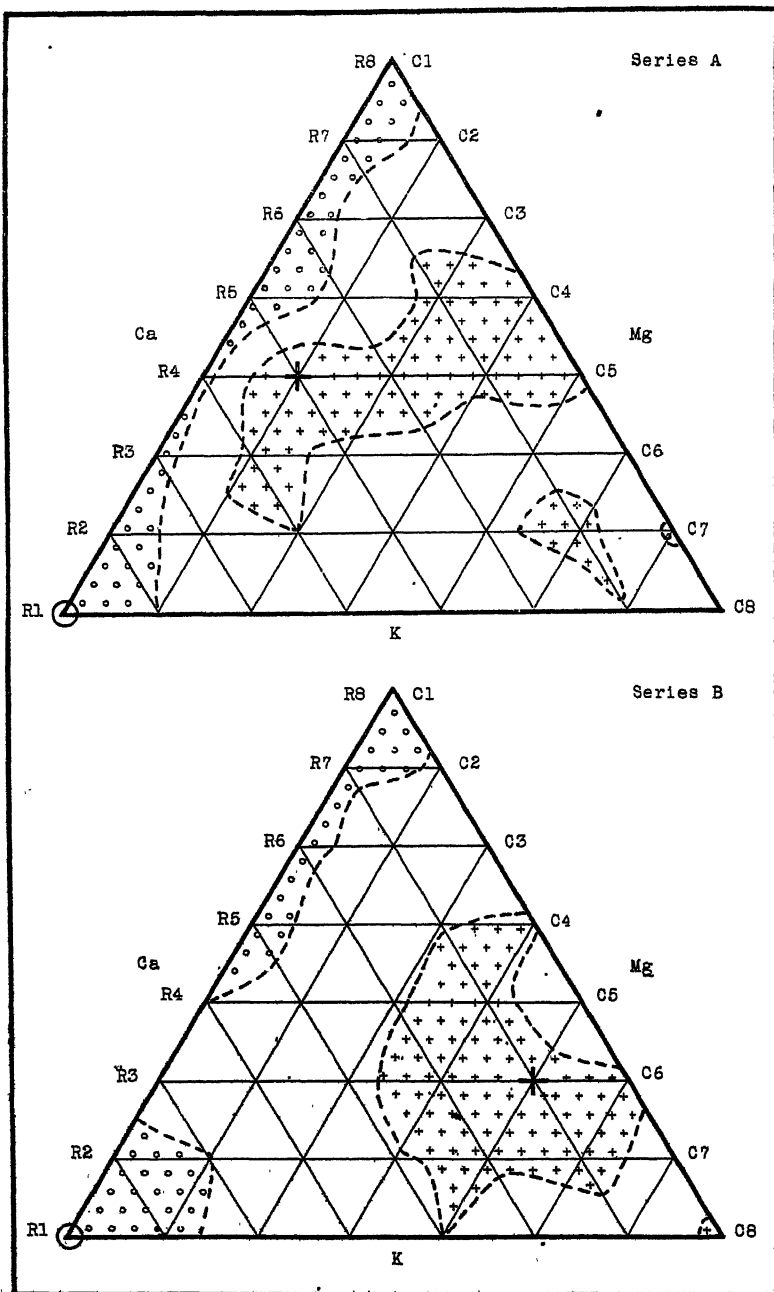


FIG. 1. DIAGRAMS SHOWING RELATIVE YIELDS OF BUCKWHEAT TOPS

Areas of high yields indicated by small crosses, those of low yields by small circles. The culture of each diagram giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

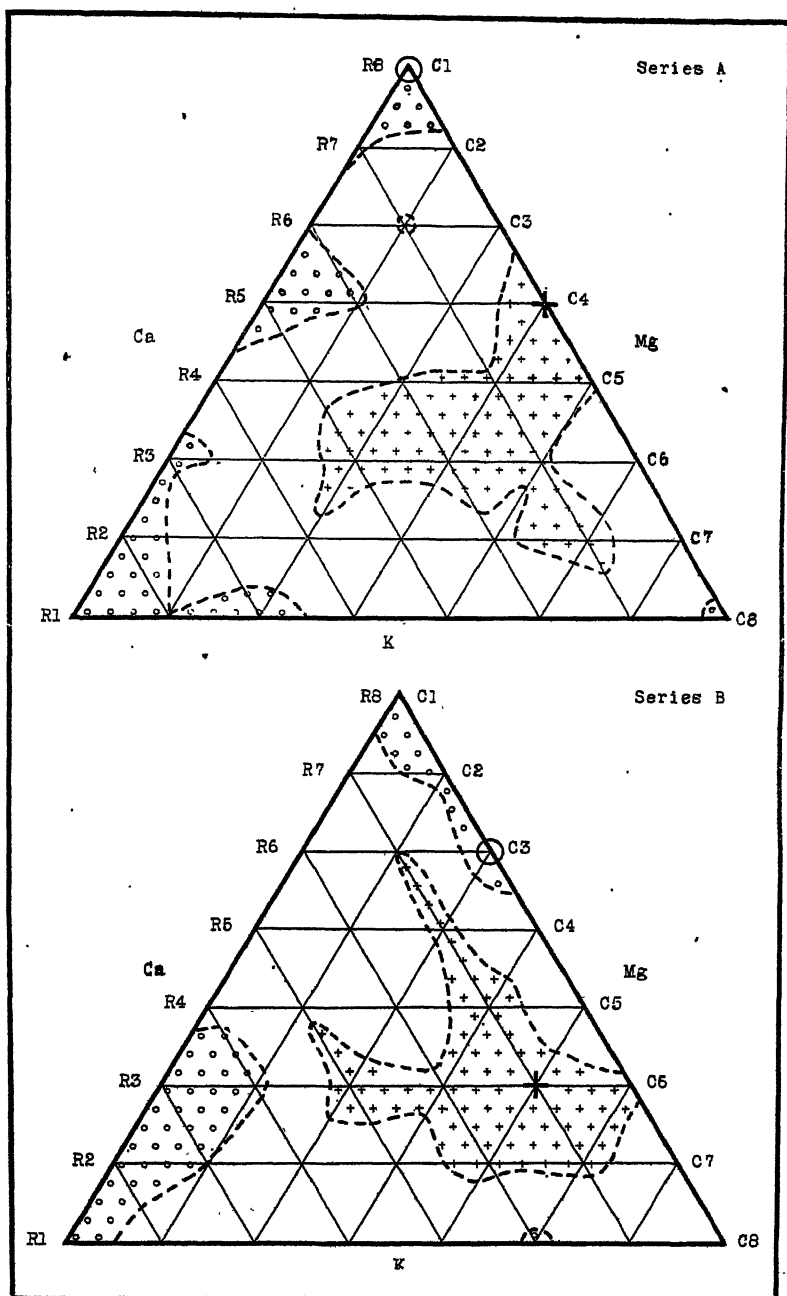


FIG. 2. DIAGRAMS SHOWING RELATIVE YIELDS OF BUCKWHEAT ROOTS

Areas of high yields indicated by small crosses, those of low yields by small circles. The culture of each diagram giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

for culture  $R_1C_1$  considered as 1.00. The highest relative yields are here indicated in black-face type. In the last column of table 3 are given the ratios of tops to seeds. The last two items in each column refer to the yields obtained from the sand cultures treated with Knop's solution and Tottingham's best solution for wheat, respectively. These were included in each series for comparison.

For facility in making comparisons, the relative yield values of series A and series B were here plotted on the triangular diagrams in the same manner as

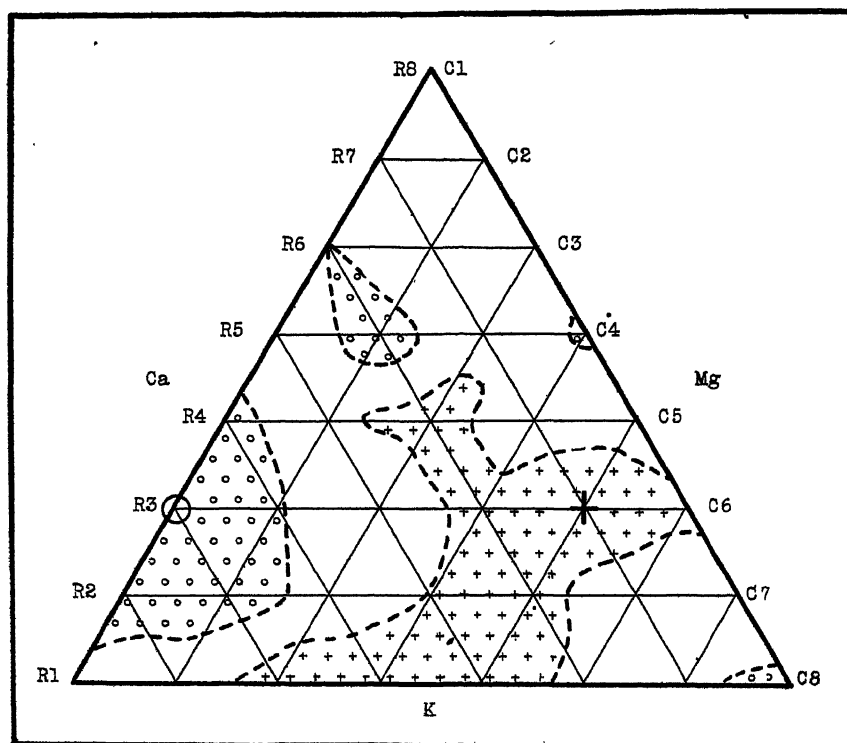


FIG. 3. DIAGRAM SHOWING RELATIVE YIELDS OF BUCKWHEAT SEEDS

Area of high yields indicated by small crosses; areas of low yields indicated by small circles. The culture giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

were the yield values obtained from buckwheat plants grown in the culture solutions of the earlier work, corresponding to the sand cultures of the present study. At the intersections of the lines showing the culture locations on the diagrams of figures 1 to 3, were placed the numbers representing the average relative dry weights taken directly from the proper columns of tables 2 and 3.

Each diagram thus graphically represents the distribution of the yield values in its respective series. In order better to study the growth rates, each series of 36 cultures was divided into three groups. One group comprises the nine cultures giving the highest yields, another group includes the nine cultures giving the lowest yields, and the remaining eighteen cultures giving medium yields comprise a third group. The positions occupied by these three groups of cultures are outlined on the diagrams of figures 1 to 3, and they correspond to the areas of high, low, and medium yields, but the yield values are omitted from the diagrams to avoid confusion. The position of any culture may readily be located on the diagram by means of its culture number, which always indicates the row and the number of the culture in the row. The rows are numbered consecutively on the left margin of each diagram, from base to apex. The culture positions in each row, represented by the intersections of the lines, are considered as numbered consecutively from left to right, the number of the last culture position in each row being given on the right margin of the diagram. The areas of high yields, corresponding to the range of the yield values for the best nine cultures, are indicated on the diagram by small crosses. The areas of low yields, corresponding to the range of the dry-weight values for the poorest nine cultures, are marked by small circles. The position on the diagram of the culture giving the *highest* yield is marked by a larger cross, and that of the culture giving the *lowest* dry weight value is shown by a larger circle.

### B. Dry weights of tops

The relations of the various salt proportions to the growth rates of the buckwheat plants during the two different developmental periods here considered, can best be compared by referring to the triangular diagrams of figure 1. The average relative dry weights of tops, as given in the third column of table 2 (series A), are here graphically represented in the upper diagram, while the lower diagram graphically represents the corresponding data of table 3 (series B). The comparisons will proceed with reference to the ranges of the high and low average dry-weight values of tops, or with respect to the extent of the corresponding areas of high and low yields as outlined on the diagrams. It is to be remembered that the position of any culture or the range of any area on these triangular diagrams, is a graphic representation of the osmotic proportions of the three salts as they occur in the solution of that culture, or of the range of these proportions in the cultures giving high or low dry weights of tops, assuming, of course, that the proportions of the salts are not altered when the solutions are introduced into the sand cultures.

1. *Early period of development (series A, fig. 1).* It will be observed that on the diagram of series A (fig. 1) the main area of low yields, including eight of the nine cultures embraced within the range of low (1.00–1.49) dry weights of tops, extends along the entire left margin of the diagram. Culture R<sub>4</sub>C<sub>7</sub> marks the upper limit of the range of low yields. The main area of high

yields (1.95-2.38) occupies a central region on the triangle, extending to the right margin at cultures  $R_4C_5$  and  $R_5C_4$ . This region includes eight of the nine cultures producing high yields of tops. A secondary high area is also indicated about culture  $R_2C_5$ .

The lowest yield of tops in this series occurred with culture  $R_1C_1$ , while the highest is shown for culture  $R_4C_2$ . The yield from this culture is 138 per cent higher than the corresponding yield from culture  $R_1C_1$ . The solution of the sand culture ( $R_4C_2$ ) producing the highest dry weight of tops is characterized by having four-tenths of its total osmotic concentration due to mono-potassium phosphate, two-tenths due to calcium nitrate, and four-tenths due to magnesium sulfate. The total range of the average relative dry weights of tops for this series extends from 1.00 to 2.38.

2. *Late period of development (series B, fig. 1).* The average dry weights of tops for the series representing the growth period between the flowering stage and maturity, range from 1.00 to 2.30, relative to the average yield from culture  $R_1C_1$ . The diagram representing the average yields of this series shows two areas of low dry weights (1.00-1.27) on the left margin, one extending to the base and the other to the apex of the triangle. The main area of high yields, embracing eight cultures, occupies a region lying principally to the right of the vertical axis of the diagram, extending to the right margin at cultures  $R_5C_4$  and  $R_5C_5$ , and touching the base of the triangle at culture  $R_1C_5$ . A secondary high area occurs also at the extreme lower right.

The lowest yield occurred with culture  $R_1C_1$ . The highest dry weight of tops was produced by culture  $R_5C_5$ . The solution of this culture is characterized by having three-tenths of its total osmotic concentration supplied by mono-potassium phosphate, five-tenths by calcium nitrate, and two-tenths by magnesium sulfate. The yield from this culture was 130 per cent higher than the corresponding yield from culture  $R_1C_1$ .

3. *Comparison of the effects of the various salt proportions upon the growth rates during the two different developmental periods. Consideration of the relative dry weights of tops (fig. 1).* From a comparison of the diagram representing the yields obtained during the early growth period (series A) with that representing the corresponding yields obtained during the late period of development (series B), it is readily apparent that there is a marked similarity between the two diagrams with respect to the positions of the areas of low top yields. Out of a total of nine cultures in each series producing low dry weights of tops, seven are included in the areas of low yields on both diagrams of figure 1. These seven cultures are included in the left marginal row, and all are characterized by low partial osmotic concentrations of calcium nitrate, but they embrace the whole range of partial concentrations of both the other salts. It thus appears that the buckwheat plants, during both the early and late developmental periods of growth, respond in much the same way to the left marginal solutions when these are supplied to the plants in a sand medium. The lowest average dry weight of tops occurred with culture  $R_1C_1$  in both series A and series B.

Turning now to the cultures which produced high yields, it is at once apparent that there is no marked similarity between the two series with respect to the positions and ranges of the areas of high dry weights of tops as outlined on the diagrams representing the two series. Of the nine cultures in each series producing high yields, three cultures only ( $R_2C_5$ ,  $R_4C_4$ , and  $R_5C_4$ ) are included in the areas of high yields on both diagrams. Culture  $R_4C_2$  in series A, and  $R_3C_5$  in series B, each producing the highest yield of tops in its respective series, are shown to occupy positions on opposite sides of the diagrams. The osmotic proportions of the three salts characterizing the former are markedly different from those which characterize the latter. The maximum yield of tops was produced during the early period of development in a sand medium provided with a solution having a higher osmotic proportion of mono-potassium phosphate, a much lower proportion of calcium nitrate, and a much higher one of magnesium sulfate than had the solution in the sand culture which gave the highest yield of tops during the late developmental period (series B). Thus, with respect to the groups of cultures producing high yields of tops, it is at once clear that the response of the plants to the proportions of the three salts in the various solutions supplied to the sand cultures, is markedly different during the two different developmental growth periods represented by series A and series B.

The readiness with which the older plants of series B respond to the variations in the proportions of the salts in the different solutions, is brought out by a comparison of the total ranges of the average relative top yields of the two series. The variations in the average relative yield values for series A extend from 1.00 to 2.38, giving a total range in these values of 1.38 from the lowest to the highest. The corresponding yield values of series B vary from 1.00 to 2.30, showing a total range of 1.30, from the lowest to the highest value. It will thus be observed that the highest yield value for each of the two series is more than double that of the lowest.

It is interesting here to compare the salt proportions producing the highest yields and the lowest yields in the sand cultures of the present study with those giving the best and the poorest yields in the corresponding series of solution cultures previously carried out. A comparison of the best sand culture of the series conducted during the early period of development (series A) with the best solution culture of the corresponding series, brings out the fact that the salt proportions of these two cultures are the same. These are the salt proportions of culture  $R_4C_2$ , the highest yield of tops occurring with this culture in each of the two corresponding series in question. The poorest sand culture and the poorest solution culture in the corresponding series carried out during the early growth period, show a marked difference in the proportions of the three salts. The former has the salt proportions of culture  $R_1C_1$ , while the latter is characterized by those of culture  $R_1C_4$ . A similar comparison of the best and the poorest physiological balance of salt proportions for the growth of buckwheat tops in sand cultures and in solution cultures carried out during



the late period of development (series B) shows that the two series agree in the proportions of the three salts required for the best yields. They agree also in the salt proportions giving the poorest dry weights of tops. In each of these two series the highest yield occurred with the salt proportions of culture  $R_3C_6$ , and the poorest yield with those of culture  $R_1C_1$ . It thus appears that the physiological properties of the nutrient solutions giving the highest yields of buckwheat tops are not greatly disturbed by the introduction of these solutions into the sand cultures, when these properties are judged by their relative effects upon the plants grown in the solutions and in sand cultures provided with the solutions. This is clearly indicated by the perfect agreement between the salt proportions characterizing the cultures giving maximum yields in the corresponding series of sand and solution cultures. Agreements similar to those here pointed out are indicated for other salt proportions characterizing cultures which produced high yields, as well as some which gave low yields, in corresponding series of sand and solution cultures, but these can best be brought out by a comparison of the triangular diagrams representing the series in question.

4. *Comparison of the ion ratio values and the ranges of these for high and for low yields of tops.* The cation ratio values and the ranges of these for the cultures giving the best nine and the poorest nine yields of buckwheat tops for each of the two series here considered, are presented in table 4. The table is divided into two vertical sections. The first column in each section gives the culture numbers and this is followed by three columns giving the ion ratio values and the total ranges of these for the best and the poorest nine cultures of the series indicated at the top. The cultures are arranged in the descending order of the magnitudes of the Mg/Ca ratio. At the bottom of the table are given the maximum and minimum values and the total range of these for the entire series. The ratio values of the culture giving the highest yield in each series are indicated in black-face type, while those of the culture giving the lowest dry weight in each series appear in italics. The cultures included in table 4 are comprised in the areas of high and of low yields outlined on the triangular diagram of figure 1.

From a comparison of the ion ratio values for the group of cultures producing high yields of tops in series A, with those of the corresponding group of series B, as given in table 4, it is at once evident that there is no substantial agreement between the two series with respect to the ranges in the values of any of the three cation ratios, Mg/Ca, Mg/K, and Ca/K. In series A the group of cultures giving high yields is characterized by a relatively narrow range in the magnitudes of each of the three ratios. The ratio ranges of this group of cultures are restricted to the lower one-third of the corresponding total ranges of the entire series. Series B, on the other hand, shows a low range (0.24 to 1.54) in the values of the Mg/Ca ratio, a medium range (0.28 to 5.55) for the ratio Mg/K, and a relatively wide range (0.58 to 5.76) in the values of the ratio Ca/K. During the early period of development, therefore, good growth of

tops was associated with Mg/Ca ratio values between 0.38 and 4.81; with Mg/K ratio values between 0.28 and 3.47; and with values of the Ca/K ratio between 0.36 and 2.16. During the late period of development good growth of tops occurred with ranges in the ratio values as follows: Mg/Ca between 0.24 and 1.54; Mg/K between 0.28 and 5.55; and Ca/K between 0.58 and 5.76.

The highest yielding cultures, R<sub>4</sub>C<sub>2</sub> in series A, and R<sub>3</sub>C<sub>5</sub> in series B, agree

TABLE 4

*Cation ratio values and ranges of these values for cultures producing high and low yields (best nine and poorest nine cultures, respectively) of buckwheat tops during the early and the late developmental periods*

	SERIES A (FIRST 4-WEEK GROWTH PERIOD)				SERIES B (SECOND 4-WEEK GROWTH PERIOD)			
	Culture number	Mg/Ca	Mg/K	Ca/K	Culture number	Mg/Ca	Mg/K	Ca/K
High yields.....	R <sub>3</sub> C <sub>2</sub>	4.81	2.32	0.48	R <sub>1</sub> C <sub>5</sub>	1.54	5.55	3.60
	R <sub>4</sub> C <sub>2</sub>	3.85	1.39	0.36	R <sub>2</sub> C <sub>4</sub>	1.44	1.39	0.96
	R <sub>3</sub> C <sub>3</sub>	3.21	3.47	1.08	R <sub>3</sub> C <sub>5</sub>	1.15	2.08	1.80
	R <sub>4</sub> C <sub>3</sub>	1.92	1.04	0.54	R <sub>4</sub> C <sub>4</sub>	0.96	0.69	0.72
	R <sub>5</sub> C <sub>3</sub>	1.28	0.56	0.43	R <sub>5</sub> C <sub>5</sub>	0.77	0.93	1.20
	R <sub>6</sub> C <sub>4</sub>	0.96	0.69	0.72	R <sub>6</sub> C <sub>5</sub>	0.64	1.39	2.16
	R <sub>7</sub> C <sub>5</sub>	0.64	1.39	2.16	R <sub>7</sub> C <sub>4</sub>	0.48	0.28	0.58
	R <sub>8</sub> C <sub>4</sub>	0.48	0.28	0.58	R <sub>8</sub> C <sub>5</sub>	0.32	0.46	1.44
	R <sub>4</sub> C <sub>5</sub>	0.38	0.35	0.90	R <sub>1</sub> C <sub>8</sub>	0.24	1.39	5.76
Range.....		4.43	3.19	1.80		1.30	5.27	5.18
Low yields.....	R <sub>1</sub> C <sub>1</sub>	15.40	11.10	0.72	R <sub>1</sub> C <sub>1</sub>	15.40	11.10	0.72
	R <sub>2</sub> C <sub>1</sub>	13.46	4.86	0.36	R <sub>2</sub> C <sub>1</sub>	13.46	4.86	0.36
	R <sub>3</sub> C <sub>1</sub>	11.55	2.78	0.24	R <sub>4</sub> C <sub>1</sub>	9.61	1.74	0.18
	R <sub>4</sub> C <sub>1</sub>	9.61	1.74	0.18	R <sub>5</sub> C <sub>1</sub>	7.70	1.11	0.14
	R <sub>5</sub> C <sub>1</sub>	7.70	1.11	0.14	R <sub>1</sub> C <sub>2</sub>	6.74	9.72	1.44
	R <sub>6</sub> C <sub>1</sub>	5.77	0.69	0.12	R <sub>2</sub> C <sub>2</sub>	5.77	4.17	0.72
	R <sub>7</sub> C <sub>1</sub>	3.85	0.40	0.10	R <sub>3</sub> C <sub>1</sub>	5.77	0.69	0.12
	R <sub>8</sub> C <sub>1</sub>	1.92	0.18	0.09	R <sub>7</sub> C <sub>1</sub>	3.85	0.40	0.10
	R <sub>2</sub> C <sub>7</sub>	0.27	0.69	2.52	R <sub>8</sub> C <sub>1</sub>	1.92	0.18	0.09
Range.....		15.13	10.92	2.43		13.48	10.92	1.35
Entire series .....	Maximum	15.40	11.10	5.76				
	Minimum	0.24	0.18	0.09				
	Range....	15.16	10.92	5.67				

in showing relatively low values for all three ratios, although there is considerable difference in the corresponding ratio values of the two cultures. The values of the three ratios Mg/Ca, Mg/K, and Ca/K for the culture giving the highest yield of tops in series A are 3.85, 1.39, and 0.36, respectively, and the corresponding values for the culture producing the highest yield of tops in series B are 0.77, 0.93, and 1.20, respectively.

A similar comparison of the ranges in ratio values for the group of lowest-yielding cultures in series A with those of the corresponding group in series B, brings out the fact that these two groups agree in showing very wide ranges in the values of the two ratios  $Mg/K$  and  $Mg/Ca$ , and a relatively narrow range in the values of the ratio  $Ca/K$ . Each of the two groups embraces the entire range of the values of the  $Mg/K$  ratio. Each group also includes the highest value of the  $Mg/Ca$  ratio and the lowest value of the ratio  $Ca/K$ .

The two series agree in showing the lowest yield for the same culture,  $R_1C_1$ . This culture is characterized by the highest value of the ratios  $Mg/Ca$  (15.40) and  $Mg/K$  (11.10), and by a relatively low value of the ratio  $Ca/K$  (0.72).

From a consideration of these data it appears that the relation between the growth rates and the ion ratio values is markedly different for the group of cultures producing high yields during the early developmental periods and the group giving corresponding yields during the late period of development. On the other hand, there is a striking similarity, with respect to this relation, between the group of low-yielding cultures of series A and that of series B.

### C. Dry weights of roots.

The average relative dry weights of roots as given in the last column of table 2, for series A, and in the fifth column of table 3 for series B, are represented graphically on the diagrams of figure 2. The upper diagram represents the data of the root yields of the young plants obtained at the flowering stage, while the lower diagram represents the corresponding data of the yields of the older plants, obtained at maturity. These two diagrams, like those of figure 1, representing the average relative yields of tops, will be compared with reference to the ranges of the yield values of the best nine and of the poorest nine cultures, and also with respect to the positions and extent of the corresponding areas of high and low yield values as these are outlined on the diagrams.

1. *Early period of development (series A, fig. 2).* On the diagram of series A (fig. 1), the low yields (0.73–1.06) are represented as occupying three areas bordering on the left margin of the diagram. Cultures  $R_1C_2$  and  $R_1C_3$  each mark the upper limit in the ranges of low-yield values. Both cultures are, therefore, included in the group of cultures giving low yields. The main area of high yields (1.41–1.65), occupies a central region on the diagram, extending to the right margin at cultures  $R_4C_6$  and  $R_5C_4$ . This area includes eight of the group of nine cultures producing high yields. A secondary high area is indicated about culture  $R_6C_2$ .

The lowest yield of buckwheat roots in this series occurred with culture  $R_3C_1$ . The highest yield is shown for culture  $R_5C_4$ . The yield from this culture is 65 per cent higher than the corresponding yield from culture  $R_1C_1$ . The solution furnished to the sand medium of this culture is characterized by having five-tenths of its total osmotic concentration due to mono-potassium

phosphate, four-tenths due to calcium nitrate, and one-tenth to magnesium sulfate. The total range in the values of the average relative dry weights of roots of this series extends from 0.73 to 1.65.

2. *Late period of development (series B, fig. 2).* The main area of low (0.78–1.07) average yields represented on the diagram of this series occupies a region bordering on the lower left margin and extends to the base of the triangle. Another low area, including three cultures, borders on the upper right margin and extends to the apex of the diagram. A small low area is also indicated about culture  $R_1C_6$ . The high (1.57–1.88) average yields of roots are represented on the diagram by a single area occupying a central region mainly to the right of the vertical axis of the diagram, and extending to the right margin at culture  $R_3C_6$ .

The lowest average yield of roots in this series was produced by culture  $R_6C_3$ . The highest yield occurred with culture  $R_3C_6$ , and was 88 per cent higher than the corresponding yield from culture  $R_1C_1$ . The solution supplied to the sand of the highest-yielding culture derived three-tenths of its total osmotic concentration from mono-potassium phosphate, five-tenths from calcium nitrate, and two-tenths from magnesium sulfate. The total range in the yield values obtained from the cultures of this series extends from 0.78 to 1.88, relative to the yield from culture  $R_1C_1$ .

3. *Comparison of the effects of the various salt proportions upon the growth rates during the two different developmental periods. Consideration of the relative dry weights of roots (fig. 2.)* A comparison of the two diagrams of figure 2, representing the yield data obtained from the young plants at the flowering stage (series A), and the corresponding data obtained from the mature plants (series B), shows the agreements and the disagreements between the two series, with respect to the distribution of the areas of low yields, to be nearly equally divided. The area of low yields at the lower left of the diagram of series A has a somewhat corresponding area on the diagram of series B. The four cultures  $R_1C_1$ ,  $R_2C_1$ ,  $R_3C_1$ , and  $R_8C_1$  are included in the areas of low yields of roots on both diagrams. With respect to the remaining areas of low yields, the two diagrams show no similarity. The lowest yield of roots in series A occurred with culture  $R_8C_1$ , while series B shows the lowest yield for culture  $R_6C_3$ .

The two diagrams of figure 2 show a certain degree of similarity with respect to the areas representing high average yields of roots. This is indicated by the fact that six of the nine highest-yielding cultures in series A appear also in the area of high root yields on the diagram of series B. The highest yields of roots, however, are shown for different cultures in the two series. The highest yield in series A occurred with culture  $R_5C_4$ . This culture is indicated as producing a low medium yield in series B. The highest yield of roots in series B is shown for culture  $R_3C_6$ . Thus the maximum yield of roots was produced during the early period of development in a sand medium provided with a solution having a higher osmotic proportion of mono-potassium phos-

phate and a lower proportion of both calcium nitrate and magnesium sulfate than had the solution in the sand culture giving the highest yield of roots during the late period of development. While it has been shown that there is a certain degree of similarity between the areas of high yields on the diagrams representing the relative dry weights of roots of series A and of series B, it is evident that there is considerable difference in the manner in which the roots of the two series responded to the variations in the proportions of the three salts. This is clearly indicated by the fact that neither the lowest nor the highest dry weights of roots occurred with corresponding cultures of the two series.

It has already been shown by a comparison of the total ranges of the average relative values of the top yields of the two series, that the older plants of series B responded quite as readily to the variations in the proportions of the salts in the solutions of the different cultures as did the young plants of series A. This is emphasized also by a similar comparison of the total ranges in the values of the relative dry weights of roots of the two series. The variations in the relative yield values of the roots of series A extend from 0.73 to 1.65, showing a total range of 0.92. The corresponding yield values of series B vary from 0.78 to 1.88, giving a total range of 1.10 from the lowest to the highest value. It will thus be observed that the range in the yield values of series B is somewhat higher than that of series A. The highest yield value for each of the two series is more than double that of the lowest, which is true also in the case of top yields.

A comparison of the diagrams of figure 2 with the corresponding ones of figure 1, brings out some interesting correlations between the growth of tops and of roots. Thus, five of the nine cultures giving high yields of tops in series A are included also in the areas of high root yields, and six cultures included in areas of low top yields appear also in the areas of low root yields. In this series, however, the *highest* yield of tops and of roots occurred with different cultures, as did also the *lowest* yield of tops and of roots.

On the diagram representing the relative yields obtained at the end of the late developmental period (series B), the main area of high top yields and that of high root yields are in very good agreement. In this series the highest yield of tops and of roots occurred with the same culture,  $R_3C_6$ . Five cultures giving low top yields also produced low yields of roots. But the lowest dry weights of tops and of roots are shown for different cultures.

4. *Comparison of the ion ratio values and the ranges of these for high and low yields of roots.* Table 5 presents the cation ratio values and the ranges of these values for the cultures giving the best nine and the poorest nine yields of buckwheat roots for each of the two periods of development here considered. This table conforms in every respect to table 4. Inspection of table 5 brings out the fact that the two series agree in showing a relatively low range in the values of each of the three cation ratios for the group of cultures in both series producing high root yields. The ratio ranges for this group of cultures in

both series are restricted to the lower one-third of the corresponding total ranges for the entire series. These ranges lie near, but do not include, the lowest values of the respective ratios occurring in the entire series. From this it appears that good growth of roots during each of the two periods of development was associated with low values of all three cation ratios. It will be observed, however, that there is considerable difference between the range values

TABLE 5

*Ion ratio values and ranges of these values for cultures producing high and low yields (best nine and poorest nine cultures, respectively) of buckwheat roots during the early and the late developmental periods*

	SERIES A (FIRST 4-WEEK GROWTH PERIOD)				SERIES B (SECOND 4-WEEK GROWTH PERIOD)			
	Culture number	Mg/Ca	Mg/K	Ca/K	Culture number	Mg/Ca	Mg/K	Ca/K
High yields.....	R <sub>3</sub> C <sub>3</sub>	2.56	1.85	0.72	R <sub>2</sub> C <sub>5</sub>	1.15	2.08	1.80
	R <sub>4</sub> C <sub>3</sub>	1.92	1.04	0.54	R <sub>3</sub> C <sub>3</sub>	2.56	1.85	0.72
	R <sub>6</sub> C <sub>2</sub>	1.92	0.46	0.24	R <sub>6</sub> C <sub>2</sub>	1.92	0.46	0.24
	R <sub>3</sub> C <sub>4</sub>	1.44	1.39	0.96	R <sub>3</sub> C <sub>4</sub>	1.44	1.39	0.96
	R <sub>4</sub> C <sub>4</sub>	0.96	0.69	0.72	R <sub>4</sub> C <sub>3</sub>	1.28	0.56	0.43
	R <sub>4</sub> C <sub>5</sub>	0.77	0.93	1.20	R <sub>2</sub> C <sub>6</sub>	0.64	1.39	2.16
	R <sub>3</sub> C <sub>6</sub>	0.64	1.39	2.16	R <sub>4</sub> C <sub>4</sub>	0.96	0.69	0.72
	R <sub>5</sub> C <sub>4</sub>	0.48	0.28	0.58	R <sub>3</sub> C <sub>5</sub>	0.77	0.93	1.20
	R <sub>4</sub> C <sub>5</sub>	0.38	0.35	0.90	R <sub>3</sub> C <sub>6</sub>	0.32	0.46	1.44
Range.....		2.18	1.57	1.92		2.56	3.71	1.92
Low yields.....	R <sub>1</sub> C <sub>1</sub>	15.40	11.10	0.72	R <sub>1</sub> C <sub>1</sub>	15.40	11.10	0.72
	R <sub>3</sub> C <sub>1</sub>	13.46	4.86	0.36	R <sub>3</sub> C <sub>1</sub>	13.46	4.86	0.36
	R <sub>5</sub> C <sub>1</sub>	11.55	2.78	0.24	R <sub>5</sub> C <sub>1</sub>	11.55	2.78	0.24
	R <sub>6</sub> C <sub>1</sub>	7.70	1.11	0.14	R <sub>2</sub> C <sub>2</sub>	5.77	4.17	0.72
	R <sub>1</sub> C <sub>2</sub>	6.74	9.72	1.44	R <sub>3</sub> C <sub>2</sub>	4.81	2.32	0.48
	R <sub>1</sub> C <sub>3</sub>	3.85	8.34	2.16	R <sub>5</sub> C <sub>1</sub>	1.92	0.18	0.09
	R <sub>7</sub> C <sub>1</sub>	3.85	0.40	0.10	R <sub>1</sub> C <sub>6</sub>	0.96	4.17	4.32
	R <sub>5</sub> C <sub>1</sub>	1.92	0.18	0.09	R <sub>7</sub> C <sub>2</sub>	0.96	0.20	0.20
	R <sub>1</sub> C <sub>8</sub>	0.24	1.39	5.76	R <sub>6</sub> C <sub>3</sub>	0.64	0.23	0.36
Range.....		15.16	10.92	5.67		14.76	10.92	4.23
Entire series .....	Maximum	15.40	11.10	5.76				
	Minimum	0.24	0.18	0.09				
	Range...	15.16	10.92	5.67				

of the ratios Mg/Ca and Mg/K in the two series, the range in the values of these two ratios for the group of cultures producing high root yields being 2.18 and 1.75, respectively, in series A, and 2.56 and 3.71, respectively, in series B. The range value of the Ca/K ratio for the group of high-yielding cultures in each of the two series is 1.92.

The cultures R<sub>5</sub>C<sub>4</sub> and R<sub>3</sub>C<sub>6</sub>, giving the highest yields in series A and B, respectively, agree in showing low values for the cation ratios characterizing

these cultures, but like the ratio ranges characterizing the groups of high-yielding cultures in the two series, the values of the corresponding ratios for the two cultures show considerable variations. Thus, the values of the three ratios Mg/Ca, Mg/K, and Ca/K for the culture ( $R_5C_4$ ) giving the highest yield in series A, are 0.48, 0.28, and 0.58, respectively, and the corresponding ratio values for the culture ( $R_3C_6$ ) producing the highest dry weight of roots in series B are 0.77, 0.93, and 1.20.

The group of nine cultures producing low yields of roots during the early period of development is characterized by the highest and lowest values of each of these cation ratios. The ranges in the magnitudes of these cation ratio values for this group of cultures are, therefore, coextensive with the corresponding ranges for the entire series. The group of nine low-yielding cultures for the late developmental period also shows very wide ranges in the values of the ratios Mg/Ca, and Ca/K, these ranges being 14.76 and 4.23, respectively. It embraces also the full range of the values of the Mg/K ratio.

From a study of the ion ratio data for high and for low yields of tops and of roots as given in tables 4 and 5, respectively, it appears that high yields of tops and of roots are, in general, associated with relatively low, but not the lowest, values of the three cation ratios. Low yields of tops and of roots, on the other hand, generally occur with solutions which are characterized by values of one or more of the three cation ratios which are either relatively high or relatively very low

#### D. Dry weights of seeds

The actual and the relative dry weights of seeds are presented in table 2 in connection with the corresponding data for tops and roots of the same series. The actual dry-weight values of seeds, as given in the table, represent the average yields from two similar series. The relative yield values were obtained in the same manner as were those of tops and of roots. The last column of table 2 gives the ratio values obtained by dividing the average actual dry weight values of tops by the corresponding values of seeds. These ratio values represent the yields of tops expressed in terms of the corresponding yields of seeds considered as 1.00.

The relative yields of seeds are graphically represented on the triangular diagram of figure 3, which corresponds to those of figures 1 and 2, representing in the same manner the relative yields of tops and of roots, respectively. It will be observed that the main area of low yields of seeds, including five of the nine cultures embraced within the range of low (0.71-0.92) dry weights, occupies a region bordering on the left margin of the diagram. Three smaller outlying areas of low yields also are indicated. The total range (1.31-1.68) of high yields of seeds is represented on the diagram by a single area occupying a central region at the base of the triangle and extending upward to the center, and to the right margin at culture  $R_3C_6$ .

The lowest yield of seeds occurred with culture  $R_3C_1$ , and the highest dry weight was produced by culture  $R_3C_5$ . The average yield from this culture was 68 per cent higher than the corresponding yield from culture  $R_1C_1$ . The total range of the average dry weights of seeds extended from 0.71 to 1.68.

A comparison of the diagram of figure 3 with those representing the yields of tops and of roots of the same series (series B, fig. 1 and 2, respectively), shows a marked degree of similarity between the diagrams with respect to the distribution of the areas of high and also of low yields. Five of the nine cultures shown in the area of high yields of seed are included in the area of high top yields, and four are also included in the area of high yields of roots. The maximum yields of tops, of roots, and of seeds were produced by the same culture,  $R_3C_5$ , but no such correlation is shown for minimum yields. The diagrams representing the three kinds of yields agree, however, in showing the main areas of low dry weights to occupy somewhat corresponding regions on the left margins of the diagrams. It thus appears that the yields of tops, of roots, and of seeds vary in a somewhat similar manner with respect to the variations in the proportions of the three salts in the solutions supplied to the sand cultures. The plants of each of the 36 cultures of this series produced an abundance of large, fairly uniform, and well filled seeds. From an inspection of the last column of table 1, giving the yields of tops in terms of the corresponding yields of seeds, considered as unity, it will be observed that these ratio values for eight cultures lie between 3.0 and 4.0, the values for twenty-three cultures lie between 2.0 and 3.0, and for five cultures the values of the ratios are between 1.0 and 2.0. The ratio of the average yield of tops to the average yield of seeds for the entire series is 2.27. This indicates that the average yield of tops for this series is only 2.27 times the corresponding yield of seeds.

A comparison of the diagram of figures 3 with the diagram graphically representing the yields of seeds from the corresponding series of solution cultures previously carried out, shows that the two corresponding diagrams are in partial agreement with respect to the distribution of the areas of high and low yields of seeds. Five of the nine high-yielding cultures in the present sand culture series are also indicated as producing high dry weights of seeds in the solution cultures, but the maximum yields of seeds did not occur with corresponding cultures of the two series. The highest yield of seeds in the sand cultures occurred with culture  $R_3C_5$ , which produced a medium yield in solution culture. Culture  $R_3C_3$ , which gave the maximum yield of seeds in solution culture, produced a medium yield in the present series of sand cultures. It is to be noted also that culture  $R_4C_3$ , which gave a high yield of seeds in sand culture, produced a low yield in solution culture, while culture  $R_5C_4$ , which is indicated as producing a high yield in solution culture, gave a low yield in sand culture. It may be said, however, that the two series show a marked similarity with respect to the position on the triangular diagrams occupied by the main areas of high and of low yields of seeds.



## *II. Effect of the sand medium upon the physiological properties of the solutions*

As previously stated, the maximum yields of buckwheat tops obtained during the early developmental period, from the present series of sand cultures, and from the corresponding series of solution cultures previously carried out, were produced by the same set of salt proportions. These were the salt proportions of culture  $R_4C_2$ . The corresponding series of sand and solution cultures conducted during the late period of development also agreed in showing their highest yields of tops for the same set of salt proportions, these being the salt proportions characterizing the culture  $R_3C_5$ . It thus appears that the physiological properties of the solutions producing maximum yields of buckwheat tops are not altered to any great extent when these solutions are introduced into the sand here employed, and in the manner described.

It is possible, of course, that solutions such as were here employed may undergo, not only a reduction in the total concentration, but also a change in the relative proportions of the constituent salts and ions, as the result of contact with the solid sand particles. It should again be emphasized, however, that in the present experiments, the sand of each culture was flooded with the nutrient solution, which was then drawn off, leaving the culture with a fixed solution content (15 per cent of the weight of the air-dry sand), after which two more portions of the solution (250 cc. to each portion) were passed through the sand cultures before the culture pots were sealed and the time period of the experiment actually begun. With such treatment and with subsequent renewal of the solutions every third day, it appears reasonable to suppose that equilibrium would soon become established with respect to the adsorptive capacity of the sand, after which the solution should suffer no further alteration from this factor, either in total concentration or in the relative proportions of the salts and ions, excepting as the adsorptive action of the sand might change with changes in temperature.

An attempt was here made to determine the influence of the sand upon the total concentration of the various solutions in the sand cultures employed, both at the beginning and at the end of an experimental period of 4 weeks' duration. For these tests the cryoscopic method was employed. A series of 36 sand cultures was prepared as already described. After the seedlings had been transplanted to the culture pots, the sand cultures reduced to the desired moisture content (15 per cent on the air-dry weight basis), and the culture pots sealed, these were allowed to stand in the greenhouse for 24 hours. A sufficient quantity of solution was then withdrawn from each sand culture, by the method already described, to be tested for the lowering of the freezing point. The solution withdrawn from each culture was replaced by an equal quantity of new solution. The cultures were then continued with renewal of solutions every third or fourth day. At the end of the growth period the solutions in the sand cultures were renewed in the usual way and the cultures were again allowed to stand for 24 hours. The cultures were then weighed

and the water lost by transpiration during the preceding 24 hours was restored by the addition of distilled water. After an interval of from 20 to 30 minutes to allow the water films to come into partial equilibrium, a small quantity of solution sufficient for the test of the lowering of the freezing point was withdrawn from each sand culture, after which the plants were harvested in the usual manner.

In table 6 are presented the results of the freezing-point determinations of the solutions withdrawn from the sand cultures supplied with the different solutions of the optimal 3-salt series employed in these studies. The solution or culture numbers referring to the triangular diagram are given in the first column of the table, which is divided into two vertical sections of three columns each. The first section presents the actual depressions of the freezing point (after corrections were made for undercooling), the osmotic concentration value at 25°C., and the variations from the original calculated osmotic concentration value of 1.75 atmospheres, for each of the 36 different solutions tested at the beginning of the growth period. The last section gives the corresponding data for the tests made at the end of the growth period. Each of the data in this table represents the average of two or more tests.

It will be observed that the osmotic-concentration values of the solutions extracted from the sand cultures at the beginning of the growth period are, with the single exception of solution  $R_7C_1$ , in very close agreement with the calculated value of the original solutions. The greatest deviation above 1.75 atmospheres is 13.15 per cent (solution from culture  $R_7C_1$ ), and the greatest below this calculated value is 4.75 per cent. The average osmotic concentration value for the entire series is 1.753 atmospheres, which represents a deviation from the original value of only 0.17 per cent. The results of the tests made at the end of the growth period show a somewhat wider variation from the original concentration value than do those of the tests made at the beginning of the growth period, the greatest deviation above the concentration value of the original solutions being 9.14 per cent, while the greatest deviation below this value is 14.28 per cent. However, the osmotic concentration values of these solutions (with the exception, perhaps, of those from cultures  $R_3C_4$ ,  $R_3C_2$ ,  $R_7C_1$ , and  $R_7C_2$ ) show as close agreement with the calculated value of the original solutions as might be expected, considering the numerous manipulations involved in the repeated renewal of solutions during a period of 4 weeks, and the chance of cumulative slight errors resulting therefrom. The average osmotic concentration value for the entire series is 1.72 atmospheres which represents a minus deviation from the calculated value of only 1.71 per cent. The majority of the solutions of this series show minus deviations from the calculated value, but many also show plus deviations, so that little significance can be attached to the comparatively slight deviations from the calculated concentration value, especially since there is no regularity in the manner in which the deviations occur.

Variations in the total concentrations such as were here observed, are scarcely sufficient to produce any marked changes in the growth rates, especially since these concentrations lie well within the range of optimal growth for these

TABLE 6

*Concentration data of solutions extracted from sand cultures at the beginning and at the end of a 4-week growth period*

CULTURE NUMBER	BEGINNING OF GROWTH PERIOD			END OF GROWTH PERIOD		
	Depression of the freezing point	Osmotic-con- centration value at 25°C.	Variation from calculated osmotic-con- centration value (1.75 atm.)	Depression of the freezing point	Osmotic-con- centration value at 25°C.	Variation from calculated osmotic-con- centration value (1.75 atm.)
	°C.	atm.	per cent	°C.	atm.	per cent
R <sub>1</sub> C <sub>1</sub>	0.134	1.76	0.57	0.135	1.77	1.14
C <sub>2</sub>	0.132	1.74	-0.57	0.132	1.74	-0.57
C <sub>3</sub>	0.130	1.72	-1.71	0.132	1.74	-0.57
C <sub>4</sub>	0.133	1.75	0.00	0.140	1.84	5.14
C <sub>5</sub>	0.134	1.76	0.57	0.123	1.62	-7.43
C <sub>6</sub>	0.131	1.72	-1.71	0.140	1.84	5.14
C <sub>7</sub>	0.131	1.72	-1.71	0.123	1.62	-7.43
C <sub>8</sub>	0.132	1.74	-0.57	0.142	1.86	6.28
R <sub>2</sub> C <sub>1</sub>	0.133	1.75	0.00	0.138	1.82	4.00
C <sub>2</sub>	0.126	1.67	-4.57	0.140	1.84	5.14
C <sub>3</sub>	0.134	1.76	0.57	0.140	1.84	5.14
C <sub>4</sub>	0.130	1.72	-1.71	0.145	1.91	9.14
C <sub>5</sub>	0.133	1.75	0.00	0.132	1.74	-0.57
C <sub>6</sub>	0.135	1.77	1.14	0.126	1.67	-4.57
C <sub>7</sub>	0.134	1.76	0.57	0.132	1.74	-0.57
R <sub>3</sub> C <sub>1</sub>	0.134	1.76	0.57	0.135	1.78	1.71
C <sub>2</sub>	0.136	1.78	1.71	0.116	1.53	-12.58
C <sub>3</sub>	0.142	1.86	6.28	0.126	1.67	-4.57
C <sub>4</sub>	0.136	1.79	2.29	0.126	1.67	-4.57
C <sub>5</sub>	0.133	1.75	0.00	0.130	1.72	-1.71
C <sub>6</sub>	0.130	1.72	-1.71	0.121	1.60	-8.58
R <sub>4</sub> C <sub>1</sub>	0.133	1.75	0.00	0.122	1.61	-8.28
C <sub>2</sub>	0.131	1.72	-1.71	0.132	1.74	-0.57
C <sub>3</sub>	0.137	1.80	2.86	0.133	1.75	0.00
C <sub>4</sub>	0.144	1.89	8.00	0.132	1.74	-0.57
C <sub>5</sub>	0.131	1.72	-1.71	0.121	1.60	-8.58
R <sub>5</sub> C <sub>1</sub>	0.134	1.76	0.57	0.133	1.75	0.00
C <sub>2</sub>	0.132	1.74	-0.57	0.132	1.74	-0.57
C <sub>3</sub>	0.135	1.77	1.14	0.132	1.74	-0.57
C <sub>4</sub>	0.132	1.74	-0.57	0.135	1.78	1.71
R <sub>6</sub> C <sub>1</sub>	0.132	1.74	-0.57	0.124	1.63	-6.86
C <sub>2</sub>	0.133	1.75	0.00	0.140	1.84	5.14
C <sub>3</sub>	0.133	1.75	0.00	0.129	1.70	-2.86
R <sub>7</sub> C <sub>1</sub>	0.151	1.98	13.15	0.114	1.50	-14.28
C <sub>2</sub>	0.133	1.75	0.00	0.114	1.50	-14.28
R <sub>8</sub> C <sub>1</sub>	0.129	1.70	-2.86	0.129	1.70	-2.86

plants, so that the results of these tests are in entire accord with the behavior of the plants in solution cultures and in the corresponding sand cultures, as this behavior was judged by the criterion of dry-top yields.

### *III. Transpiration and water requirement*

Since transpiration may be regarded as a valuable indicator of the general health and vigor of plants, it was deemed worth while to compare the relative amounts of water lost from the various cultures during their growth period with the data of the other plant measurements. From the relation between the amounts of water lost by transpiration and the dry-weight yields of tops, roots, and seeds, may be derived also the ratios representing the amount of water lost for each single gram of dry plant substance produced. These ratios of transpiration to yields represent the water requirements of the plants. Because of the importance of this criterion of plant growth, and for the sake of completeness, it seemed desirable also to compare these derived data with the direct quantitative plant measurements.

The data of transpiration and water requirements are presented in table 7 in three sections. The first section gives the relative amounts of water loss from the cultures of the two series representing the two different developmental periods here considered. This is followed by the section giving the water requirements of tops and of roots for series A. The last section presents the corresponding data for series B, and also the water requirements of seeds for this series. The various data for each culture are expressed in terms of the corresponding data for culture  $R_1C_1$  in the respective columns. In each column the actual value for this culture is given in parentheses just below the relative value.

The average data of table 7 were plotted on triangular diagrams in the same manner as were the yields of tops, roots, and seeds, and the corresponding diagrams of the two different series thus obtained were compared with each other and also with the corresponding yield diagrams already given (figs. 1, 2 and 3). The various diagrams graphically representing the data of table 7 are not here presented, but the main points of interest brought out by these comparisons will be given in brief.

#### **A. Relation of transpiration to yields**

The main areas representing high and low water loss on the transpiration diagram of series A occupy positions corresponding very closely with those occupied by the main areas of high and low yields, respectively, of tops and of roots on the yield diagrams. The highest total amount of water loss from a single culture of this series, however, did not occur with the same culture giving the maximum yield of tops, nor with that producing the highest yield of roots. In series B the agreement between the transpiration diagram and the corresponding yield diagrams of tops and of roots, with respect to the

main areas of both high and low yields, is even more pronounced than it is in series A. The maximum yield of tops and of roots and the greatest amount

TABLE 7

*Data of transpiration and water requirement; series A grown to the flowering stage, series B grown from the flowering stage to maturity in sand cultures supplied with 3-salt solutions*

CULTURE NUMBER	TRANSPIRATION		WATER REQUIREMENT				
	Series A	Series B	Series A		Series B		
			Tops	Roots	Tops	Roots	Seeds
R <sub>1</sub> C <sub>1</sub>	1.00 (502)	1.00 (2384)	1.00 (254)	1.00 (1716)	1.00 (485)	1.00 (4599)	1.00 (794)
C <sub>2</sub>	1.21	1.59	0.83	1.21	1.25	1.45	1.32
C <sub>3</sub>	1.31	1.68	0.89	1.50	1.10	1.01	1.23
C <sub>4</sub>	1.36	1.81	0.83	1.26	1.07	1.17	1.16
C <sub>5</sub>	1.42	1.81	0.89	1.29	0.98	1.12	1.21
C <sub>6</sub>	1.49	1.64	0.90	1.28	1.04	1.22	1.50
C <sub>7</sub>	1.40	1.60	0.77	1.26	0.93	0.98	1.66
C <sub>8</sub>	1.35	1.66	0.91	1.66	0.89	1.22	2.04
R <sub>2</sub> C <sub>1</sub>	1.06	1.49	1.00	1.35	1.13	0.97	1.96
C <sub>2</sub>	1.53	1.32	0.85	1.19	1.01	1.15	2.14
C <sub>3</sub>	1.59	1.62	0.86	1.35	0.95	0.90	2.17
C <sub>4</sub>	1.41	1.73	0.80	1.32	0.93	0.86	1.41
C <sub>5</sub>	1.68	1.89	0.92	1.26	0.99	0.85	1.38
C <sub>6</sub>	1.86	1.79	0.93	1.45	0.93	0.91	1.36
C <sub>7</sub>	1.48	1.59	1.04	1.35	0.89	1.05	1.44
R <sub>3</sub> C <sub>1</sub>	1.26	1.60	1.01	1.30	0.96	1.20	2.98
C <sub>2</sub>	1.49	1.86	0.89	1.39	1.01	1.65	1.61
C <sub>3</sub>	1.62	1.38	0.88	1.10	0.73	0.62	1.40
C <sub>4</sub>	1.59	1.72	0.89	1.42	0.85	0.85	1.25
C <sub>5</sub>	1.55	2.03	0.86	1.43	0.78	0.78	1.17
C <sub>6</sub>	1.49	1.84	0.93	1.36	0.94	0.89	1.26
R <sub>4</sub> C <sub>1</sub>	1.39	1.46	0.98	1.16	1.04	1.09	1.90
C <sub>2</sub>	1.72	1.77	0.76	1.33	1.07	0.88	1.40
C <sub>3</sub>	1.61	1.83	0.80	1.26	1.03	0.99	1.35
C <sub>4</sub>	1.71	1.81	0.89	1.23	0.88	0.77	1.77
C <sub>5</sub>	1.69	1.61	0.89	1.28	1.15	1.06	1.98
R <sub>5</sub> C <sub>1</sub>	1.26	1.69	1.10	1.55	1.47	0.96	1.40
C <sub>2</sub>	1.33	1.77	0.89	1.35	1.11	0.87	1.55
C <sub>3</sub>	1.74	1.85	0.87	1.63	1.01	0.87	1.80
C <sub>4</sub>	1.50	1.61	0.83	0.99	0.85	0.94	1.78
R <sub>6</sub> C <sub>1</sub>	1.21	1.44	0.99	1.20	1.26	0.93	2.07
C <sub>2</sub>	1.48	1.71	0.84	1.07	1.04	0.88	1.55
C <sub>3</sub>	1.51	1.75	0.85	1.23	1.04	1.61	1.64
R <sub>7</sub> C <sub>1</sub>	1.20	1.64	0.97	1.22	1.27	1.32	1.54
C <sub>2</sub>	1.34	1.64	0.90	1.26	1.09	1.06	1.59
R <sub>8</sub> C <sub>1</sub>	1.24	1.65	0.95	1.26	1.32	1.30	1.70
K	1.50	1.76	0.92	1.36	0.97	0.85	1.30
T	1.64	1.72	0.91	1.23	1.01	1.05	2.03

of transpirational water loss from any single culture here occurred with the same culture,  $R_3C_5$ . The lowest yield of tops and the smallest amount of water loss is shown for culture  $R_1C_1$ .

From these considerations it is clear that high transpiration, in general, is associated with high yields of tops and of roots, and low transpiration with low yields, an observation which is in entire accord with what has already been found in the study of buckwheat in solution cultures corresponding to the present study of these plants in sand cultures.

A comparison of the transpiration diagram of series A with the corresponding diagram of series B, brings out the fact that there is no agreement between the two diagrams with respect to the areas of high transpiration values. The two series agree, however, in showing the main regions of low transpiration as bordering on the left margins of the diagrams. It thus appears that the relation between the various salt proportions and transpiration, with respect to the two different periods of development, is much the same as is the relation between the salt proportions and the yields of tops and of roots.

#### B. Relation of water requirements to yields

A comparison of the water-requirement diagrams with the corresponding yield diagrams, shows that the relations between water requirement of tops and the dry-weight yields of tops are very well defined in each of the two series here considered. On the diagram representing the water requirements of tops of series A, the areas of high values agree absolutely with the areas of low values on the corresponding diagram of top yields, while the regions of low water requirements agree in a general way with those of high top yields. There is no detailed relation, however, between the water requirements of roots and root yields in this series.

On the diagrams of series B, the regions of low water requirements correspond very closely with those of high top yields, while the areas of high water requirements and low top yields show equally good agreement. There is a marked tendency also toward the same relations between the water requirements of roots and root yields in this series, although the agreements are not so exact as they are in the case of the water requirements of tops and top yields.

A comparison of the water requirement diagram for seeds with the corresponding yield diagram shows the areas representing low water requirements to correspond very closely with those of high seed yield, while the regions of high water requirements of seeds are in close agreement with those of low seed yields. Thus, with the sand cultures of the present study, as with the corresponding solution cultures of the earlier work, low water requirements are associated with high yields of tops, roots, and seeds, and high water requirements correspond to low yields.

From a comparison of the corresponding diagrams representing the water

requirements of the two different series, it is clear that there is as little correlation between the water requirements of the young plants of series A and the older ones of series B as there is between the dry-weight yields, either of tops or of roots, of the two different series, or between the transpirational water loss from the cultures of series A and those of series B. Thus, by whatever set of measurements the relation between the growth rates and the proportions of the salts in the media is judged, this relation is found to be markedly different for the two different developmental periods.

In the earlier work with solution cultures corresponding to the present series of sand cultures, it was emphasized that the changes in the physiological requirements of these plants, with respect to the proportions of the salts in the nutritive media might be a gradual process extending over a comparatively long interval of time, involving perhaps the entire life period of the plants. On the other hand, it was pointed out that the change in salt requirements of the plants may take place comparatively rapidly with the marked changes which occur within the plants during the blossoming stage, when the vegetative processes become less active and the reproductive and seed-forming processes begin.

In some recent work by McCall (6), the active life period of the wheat plant was divided to cover three stages in its development: the first 30-day period, the second 30-day period, and finally the period extending from the close of the second 30 days to the maturity of the plant. McCall was able to show that the mineral requirements of the wheat plant during the first and the second 30-day periods were substantially the same, but the salt proportions producing high and low yields during the third growth period were markedly different from those giving corresponding yields during the first and second 30-day growth periods. While this does not directly apply to the salt requirements of the buckwheat plants here employed, it strongly suggests the possibility that the change in physiological requirements of these plants may take place during the flowering stage or during the period extending from the flowering stage to the maturity of the plants.

#### SUMMARY

The preceding pages present the results of a comparative study of the salt requirements of buckwheat plants during two different developmental periods. The plants were grown in sand cultures supplied with nutrient solutions of the same initial total osmotic concentration value of 1.75 atmospheres, but differing in the proportions of the component salts. The series of solutions supplied to the sand cultures comprised 36 different sets of salt proportions of the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ .

The results obtained from the plants grown during the first 4 weeks after germination, in sand cultures supplied with nutrient solutions, were compared with those obtained from older plants grown during the period of development

between the flowering stage and maturity, in sand cultures supplied with the same solutions. The main facts brought out by this comparative study are briefly summarized as follows:

1. The highest yield of buckwheat tops obtained in a period of 4 weeks directly following germination occurred with the sand culture supplied with a solution having the following salt proportions:  $\text{KH}_2\text{PO}_4$ , 0.0144 m.;  $\text{Ca}(\text{NO}_3)_2$ , 0.0052 m.; and  $\text{MgSO}_4$ , 0.0200 m. The highest yield of tops, of roots, and of seeds was obtained, during the second developmental period, from a sand culture supplied with a solution having the salt proportions of  $\text{KH}_2\text{PO}_4$ , 0.0108 m.;  $\text{Ca}(\text{NO}_3)_2$ , 0.0130 m.; and  $\text{MgSO}_4$ , 0.0100 m. Thus the maximum yields were produced during the late developmental period in a sand culture furnished with a solution characterized by having a lower proportion of mono-potassium phosphate, a much higher proportion of calcium nitrate and a much lower one of magnesium sulfate than had the solution supplied to the sand culture giving the highest yield of tops during the early period of development.

2. The salt proportions of the solution in the sand culture of the present series producing the maximum yield of tops during the early developmental period, and those giving the highest yields of tops and of roots during the late period of development, are in exact agreement with those giving maximum yields of tops and of roots in the corresponding series of solution cultures previously carried out.

3. Under the conditions of these experiments the physiological properties of the nutrient solutions, as these affect the growth of the plants, are not altered to any marked extent when the solutions are added to the sand cultures.

4. High yields of tops and of roots are, in general, associated with relatively low values—but not the lowest values—of the three cation ratios  $\text{Mg}/\text{Ca}$ ,  $\text{Mg}/\text{K}$ , and  $\text{Ca}/\text{K}$ . The values of these ratios characterizing the solutions supplied to the sand cultures, show pronounced differences for the cultures giving maximum yields of tops, and maximum yields of roots, and also for those giving minimum yields of roots, during the two different developmental periods of growth.

5. High transpirational water loss is associated with high yields of tops and of roots, and low transpiration with low yields

6. For each of the two developmental periods of growth, high water requirement is, in general, associated with low yields of tops and of roots, and low water requirement with high yields.

7. The relation of the growth rates of the buckwheat plants to the variations in the osmotic proportions of the solutions supplied to the sand culture is markedly different for the two different developmental periods of growth, whether this relation is judged by the criterion of tops or of roots, by that of transpiration, or by that of the water requirements of tops or of roots.



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# SOIL ACIDITY METHODS

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## I. A COMPARISON OF SEVERAL METHODS

### *Introduction*<sup>1</sup>

It is questionable whether any method now in use gives correctly the absolute acidity in the soil, and probably since there is such a wide variation in results, most of them do not even approximate it. But even though total acidity cannot be measured accurately, it is very desirable that reliable comparative results should be obtained. The first problem in the study of soil reaction, therefore, is the selection and standardization of a method. This same problem is met in many studies, but probably never more emphatically than in an investigation of the nature and significance of soil acids.

In this work, the study of methods has been undertaken only as preliminary to other investigations, the object being merely to select a method suitable for use. The methods taken up are typical of those now employed in soils work, and include the Hopkins, Veitch, Jones, MacIntire, Truog and Tacke methods. Many modifications of these more or less standard methods have been devised by various workers, but the consideration given here covers the principles involved in the operation of the methods in general.

### *Experimental procedure*

*Hopkins.* A rather popular method is that put forward by Hopkins (2) of Illinois. Results here depend upon the liberation of acid from a neutral salt, or upon an exchange of bases freeing iron and aluminum from zeolites or other minerals, which action amounts to the same as that first mentioned, namely, a free acidity. The action is brought about by shaking the acid soil and a salt together for three hours. A solution of common salt was originally used. Later potassium nitrate was found more reactive and substituted for the salt. In this work sodium nitrate has been employed but the action is probably approximately the same.

<sup>1</sup> This opportunity is taken to extend acknowledgments to Dr. R. S. Potter for the use of his laboratory and for helpful consultations during the progress of the work; also to Dr. P. E. Brown for suggestions and criticisms offered at various times.

*Jones.* The Jones method (3) is similar in type. In this case calcium acetate is used, and the application is made dry, mixing primarily by trituration, though solution and more intimate contact occurs when water is added. The two methods, though based upon identical principles, give a wide difference in the lime requirement as shown by the tests.

*MacIntire.* The procedure (4) used in this method consists of treating the soil with N/28 calcium bicarbonate, and evaporating to a thin paste on a steam bath. During this process the bicarbonate unites with and neutralizes the soil acids.

*Veitch.* This method (9) makes use of N/28 calcium hydroxide to neutralize the soil acids, and in the treatment which indicates the lime requirement there is no excess of base present. In this work, to facilitate speed, the first evaporation was carried out on a hot plate until there was danger of spattering, finishing on a steam bath.

*Truog.* For this test (8) 4/10 normal barium hydroxide is used to neutralize the acidity. The acid soil and base are allowed to react for just one minute, during which time the active acidity is supposedly neutralized. The mixture is then evaporated to dryness on a water bath, before determining the excess of base employed.

*Tacke.* The Tacke method (6) makes use of pure calcium carbonate and water to bring about the neutralization of soil acids. The original technique was used except for such changes as are mentioned later in the discussion. No heat is employed, but intimate contact between the soil and lime is secured by vigorous and continuous shaking. As the reaction occurs, carbon dioxide is evolved and removed by aeration. The amount of carbon dioxide evolved determines the amount of acidity.

### *Discussion of results*

In these studies two soils which are designated as brown silt loam and gray silt loam have been used with all the methods. The names given are not presumed to describe the soils accurately, but the distinction is sufficiently exact for practical work.

The summary table presents some interesting facts. For the sake of comparison the lime requirement indicated by the Tacke method is given the value 100, and the relative value is recorded for the other methods. The results are expressed in pounds of calcium carbonate per 2,000,000 pounds of soil.

It is observed that the Veitch method gives results which although rather inconsistent agree fairly well with those secured by the Tacke method and that none of the other methods agree very closely on either soil. This is in favor of the Tacke procedure, since the Veitch determination, even if not entirely reliable, should more nearly indicate the approximate neutral point than those methods where strong bases or a preponderance of carbonate is

used at high temperatures. In other works the modified Tacke method gives as nearly the true lime requirement and is much more consistent than any other test tried.

Another noticeable thing is that the methods do not vary in the same way with the different soils. This is indicative of a difference in reactivity of the acids of the two soils. It emphasizes, likewise, the unreliability of quantitative indications as well as the difficulties of accurate qualitative studies, where very different soils and treatments are employed. But a consideration of the nature of soil acidity leads to the prediction of such results.

True acidity has been defined as a hydrogen-ion concentration greater than that of pure water. In other words, regardless of how it is produced, there is no other acidity, in the soil or elsewhere, than hydrogen-ion. Hydrogen-ion concentration must be, therefore, the predominating factor in determining the injury which soil acids may cause to crops, either directly or indirectly,

TABLE 1  
*Table showing the lime requirement by the different methods on two soils*

METHOD	LIME REQUIREMENT			
	Gray silt loam		Brown silt loam	
	Pounds per acre	Value	Pounds per acre	Value
Tacke (10 hr.).....	4300	100.0	6500	100.0
Hopkins.....	2000	46.5	2400	36.9
Jones.....	3857	89.7	4821	74.2
MacIntire.....	3675	85.4	4070	62.6
Veitch.....	4648	108.1	6330	97.4
Truog.....	12200	283.7	15940	245.2

through their action upon soil microorganisms. Any poisoning which may be due to other specific ions is not here considered an acidity phenomenon. Recognizing this as the correct point of view, it becomes evident that there may be a high potential acidity without an injurious concentration of hydrogen-ion. As an example, a soil which contains sand has a potential acidity, represented by all the sand present. To how great an extent the silicon dioxide may be hydrolyzed to silicic acid in the soil cannot be determined, and to what extent this reaction may occur during the process of measuring the acidity by some of the common methods, it is likewise at present impossible to calculate. With confidence, it may be said that such reactions take place slowly under field conditions. In the field, too, reverse reactions must occur, in the dehydration of the silicic acid and the carbonating of calcium silicate. These reactions, likewise, must usually be slow.

But, nevertheless, in the soil even the salt calcium silicate represents also a potential acidity. If, when nitric acid is produced by the nitrifying organisms,

it reacts as it might with calcium silicate, soluble calcium nitrate would be produced, some of which would be removed by leaching, leaving relatively insoluble silicic acid. This reaction, too, must occur slowly and only to a limited extent under normal conditions. Though this reaction would furnish nothing but relatively inactive acid, which may not be directly harmful, the results are quite different when aluminum or iron salts are liberated. To what extent these reactions occur is also somewhat a matter of speculation though some investigations have been made to determine the iron and aluminum liberated by neutral salts. Since this work has been only a test of methods, other phases of the acidity problem have been investigated only in a very limited way, and little can be said in regard to the specific problems of acidity except as they have been brought out in the general study. It is very probable, however, that some of these potential acids affect the lime requirement as indicated by the different methods, and sufficient data are at hand to present some pertinent criticisms of the methods tested.

### *Objections to the methods*

*Hopkins vs. Jones.* Since these two methods are so much alike they are discussed together. A wide difference between them is shown, the Jones method giving more than three times as high a lime requirement as the Hopkins. A different factor has been used at different times for the Hopkins method, the highest one which has been noted being 4. But even this factor ( $2\frac{1}{2}$  was used in the calculations) does not make the requirement at all comparable with that indicated by the use of calcium acetate. It should not, however, be expected that the two methods would agree. Jones with his method uses no factor, assuming that the reaction gives the correct lime requirement. Since nitric and acetic acids do not have strengths of the same order of magnitude, equivalent amounts of each would not be set free by the soil acids. Then too, there is the difference in solubility of the respective salts of the soil acids with sodium and calcium. Since calcium salts, as a rule, are much less soluble, there will be a more complete displacement of the acid from calcium than from sodium salts. It is logical, therefore, that a higher result should be obtained with the calcium salt of a weak acid than with the sodium or potassium salt of a strong acid. That the Hopkins procedure would give different results with different salts used in making the extraction also is without question.

Finally, since the effective strength of acids depends upon solubility and ionization, and since soil acids may possess greatly varying effective strengths, it is not probable that soils with the same potential acidity would give at all comparable hydrogen-ion concentrations. The amount of acid liberated from a neutral salt therefore, would be quite variable with different soils for a single extraction, and the factor used should vary accordingly in order to adapt

the method to all soils. To secure a variable factor of this type that would operate accurately would be impossible. An acid soil which gives a high hydrogen-ion concentration would require fewer extractions to remove the total acidity and, therefore, a smaller factor. A very unreactive acid would be extracted only with difficulty, if at all. The data given here indicate, however, that a considerable part of the soil acids of normal soils is sufficiently active to be obtained with comparative ease. This must evidently vary with soils, and though the method is hardly sensitive enough for such work, results show that acidity curves diverge at first and then finally tend to come together, these curves representing the rate of evolution of carbon dioxide which is the index of reactivity of the soil acids.

The conclusion seems justified, therefore, that neither of the above procedures can give results which are reliable and consistent when different soils and treatments are studied. But it is possible that many methods may be sufficiently accurate to indicate the amount of lime to apply to farm land. The farmer does not usually apply small fractions of tons and the distribution

TABLE 2

*Lime requirement in pounds of  $\text{CaCO}_3$  per 2,000,000 pounds of soil by the MacIntire method with varying amounts of soil*

SOIL	LIME REQUIREMENT		
	20 gm.	10 gm.	5 gm.
Brown silt loam.....	5,175	8,350	10,700
Soil blank.....	450	300	150

and mixing with the soil cannot be at all uniform. The recommendations made to him, too, are as a rule in excess of the indicated requirement when it is low, but below it when it is high. That a rough method which might serve this purpose would not be satisfactory for research work is quite evident.

*MacIntire.* The determinations by this method do not agree with the two previous results, though when the original directions were followed (except that a 20-gm. sample was used) they were rather close to those given by Jones' method. However, when tests were made with varying amounts of soil, a soil blank being used, the results given in table 2 were obtained.

The same amount of bicarbonate is used in each case, the amount of soil being the only variable. It is noted that the lime requirement depends very much upon the amount of soil used. When there is a large excess of bicarbonate, nearly double the amount is taken up, as a result of the influence of mass action. Hydrolytic reactions may exert a greater effect also. Since calcium hydroxide is soluble to the extent of one part in six hundred of water, more soluble than the carbonate or bicarbonate, and is a strong base relative to its concentration, hydrolysis would increase the action of the carbonate

upon the soil. These variations are sufficient objection to throw the method into disrepute. Ames and Schollenberger (1) in their work on different methods show that this method is very sensitive to variations in manipulation. The directions call for evaporation to a thin paste. This is a very indefinite end point, and other workers have found that both the degree of dryness and the rate at which it is evaporated cause a wide variation in the lime requirement. The same result is observed in this work in the two determinations. The increase in lime requirement with length of time of contact between moist soil and bicarbonate would indicate that an equilibrium is reached rather slowly. A large excess of lime with a small sample of soil would permit the attainment of equilibrium sooner and would be one cause of the higher requirement. The amount of organic matter decomposed also is greater when a longer time is allowed before the mixture of acid soil and lime is brought to complete dryness.

TABLE 3

*Determinations of lime requirement in replication by the Veitch method, expressed as tons of  $\text{CaCO}_3$  per 2,000,000 pounds of soil*

NO.	LIME REQUIREMENT	
	Gray silt loam	Brown silt loam
	tons	tons
1	2.5	3.57
2	2.14	3.21
3		3.03
4		2.85

*Veitch.* Several tests were made by this method in an attempt to duplicate the determinations. Often not only did the results disagree, but either all soil treatments were alkaline or none were alkaline, and the test was apparently entirely outside the range of acidity. Never less than two treatments on each side of the previous approximately neutral indication were used. This gives a range of 1785 pounds, but many times it proved inadequate. Some characteristic variations are given.

As to the causes for the variations, there may be several. Some action, such as hydrolysis and the effect of organic matter and soil particles upon the indicator, have already been suggested by other workers (1). The rate at which the evaporation is carried out on the hot plate is a source of marked variations in the results as found in this work. When taken down rapidly (about 30 minutes on the hot plate) it is impossible to read the end point accurately. The rapid heating seems to deflocculate the soil colloids and the minute particles never settle out. When taken down slowly (about  $1\frac{1}{2}$  hours on the hot plate) this trouble does not occur, and a nearly clear liquid for the final reading may be obtained. It should be understood that the evaporation

is finished on a steam bath and there is no cooking of the soil from excessive heat.

The rate of evaporation on the water bath, however, seems to have less effect. But this rate cannot be augmented much since a temperature higher than that of steam cannot be obtained. Readings from the final evaporation are sometimes very difficult even when the liquid is quite clear. At the Ohio Station (1) filtering was resorted to in order to remove soil particles. These many variations in procedure must give decidedly different results. And what is worse, perhaps, it seems impossible to standardize the operations. As an example of lack of uniformity it may be pointed out that the final evaporation is a very indefinite process. The reading depends considerably upon the degree of dryness obtained and it is impossible to go always to the same point. Directions call for taking down to about 15 cc. in a 100-cc. beaker. The best guessing would probably vary at least 50 per cent from this amount, with a somewhat proportionate effect upon the reading. And too, there are the many ill effects which may be attributed to the application of heat to the soil during the determination.

After considerable work, double distilled neutral water being used part of the time to remove all source of error due to impure water, it was concluded that not only can one worker not duplicate the results of another by this method, but that the same worker may not obtain concordant results except as an accident. The method, therefore, cannot be considered suitable for accurate experimental work. The presence of large amounts of calcium carbonate which has been added to the soil in some of the work would also probably interfere with the successful use of the method.

*Truog.* The requirements indicated by this method are much higher than those given by any of the other determinations. It has been shown in another paper that this method is susceptible to an effect from varying the relative amounts of soil and alkali and from the dilution used when adding the alkali to the soil. Also, different bases give different results. The procedure seems, therefore, unreliable and gives an unreasonably high lime requirement partly because of mass-action effects. Since one of the products of the reaction of the base with the soil acids is water, which has the same effect as removing one of the products of the reaction, the equilibrium must be pushed far in that direction, and also the strong alkali may decompose organic material in the soil, and thereby indicate an unduly high acidity. Normally the decomposition of organic materials may occur slowly enough that most of the acids produced are oxidized as rapidly as they are liberated. Mineral acids are apparently the only source of a stable soil acidity. In experiments by Temple (7) where organic acids are added to the soil they were found to disappear completely in two weeks. Bases held as organic salts are, therefore, likely to be freed to act again in neutralizing the more permanent acids. Partly, for this reason, soils high in organic matter are less harmfully affected by a given degree of acidity.



### Conclusion

After a preliminary test of the various methods, the results seem to indicate that one method only is reliable for research work. This is a modification of the old Tacke procedure quite popular in Europe, but as yet little used in this country. Accordingly, this method was taken up in an attempt to develop it to a higher degree of efficiency.

## II. TACKE METHOD

### *The general action of methods*

There must always be severe criticism to offer for any method of determining soil acidity where a strong base or heat is employed in the determination. Heat increases hydrolytic reactions and augments errors of this source. Heat, too, decomposes organic matter, as do also strong bases, therefore, indicating an unduly high and often inconsistent lime requirement. A modification of the Tacke procedure which originated at the Ohio Station (1)

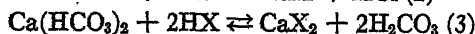
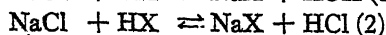
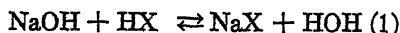
TABLE 4

*Lime requirement by the Tacke method, comparing pure water and saturated CaCl<sub>2</sub>, and varying the time of running*

TREATMENT	LIME REQUIREMENT			
	3 hrs.	6 hrs.	9 hrs.	21 hrs.
	lbs.	lbs.	lbs.	lbs.
100 cc. pure H <sub>2</sub> O and 2 gm. CaCO <sub>3</sub> .....	5,000	6,100	6,500	7,000
50 cc. saturated CaCl <sub>2</sub> and 2 gm. CaCO <sub>3</sub> .....	3,900	5,150		5,475

and which we have not tried, makes use of heat in a partial vacuum to hasten the reaction. In this manner a temperature no higher than 50°C. may be maintained, thereby reducing to a minimum the decomposition of organic matter by heat. Nevertheless, the authors report a high lime requirement, and it has seemed advisable to lengthen the time of contact between soil and carbonate rather than attempt to gain speed by the use of heat.

The end point of the various reactions occurring with the different methods used depends very much upon solubilities. Three type reactions are given:



Here *HX* may represent any soil acid. It is not necessary that the *X* represent any particular valency except for the sake of giving balanced equations. In fact *X* might as well stand for a very complex acid radical. The principle involved remains the same. It is quite evident that in reactions such as shown

in equation (1), where water is one product, the equilibrium is far removed to the right. The reaction occurs rapidly because of the solubility and strength of the base used. A very insoluble salt would produce the same effect on the equilibrium as the water. It is just as evident that reactions similar to that shown in equation (2) can not go far to the right. Solubility and ionization of the reaction products on either side of the equation must be considered in the equilibrium systems. Of course soil reactions are not so simple as this but the results are illustrative of the principles involved. In equation (3) is shown the reaction which must occur with the use of the Tacke method. The removal of the carbon dioxide must carry this equilibrium far to the right according to the mass law. The reaction proceeds slowly, however, because of the slight solubility of both the base and the soil acids.

Perhaps there is no strong argument for using the same materials in a determination of soil acidity as are used in correcting this condition in the field. In fact, there are many bases in the soil which have a neutralizing power. Yet, there is no proof that finely-divided calcium carbonate brought into intimate contact with the acid soil by vigorous shaking, mixing, and aerating, should not give the true lime requirement. The results of this method with the various modifications tested are presented below.

### *Procedure*

The method (6) was designed originally for European moor soils, high in organic matter. The soils used here, however, are mineral, but the method has proved no less satisfactory with them. Previously in this country the procedure has been tried to some extent by Wheeler, Hartwell, and Sargent (10). No one, however, has ever made use of the vigorous shaking which is emphasized here.

The procedure consists in treating the soil directly with calcium carbonate in 500-cc. Kjeldahl flasks, using a 20-gm. sample of soil and about 2 gm. of carbonate, with 50 cc. of carbon-dioxide-free water. The carbonate is introduced as a water suspension. The limestone reacts with the soil acids and the carbon dioxide thus liberated is collected in sodium hydroxide towers. The absorption towers are cylinders holding about 150 cc. and the sodium hydroxide, of which 100 cc. is used, has a strength of approximately 2.5 per cent. Two rubber discs in the cylinders and traps on top aid in the absorption of the carbon dioxide. The machine provides for 10 Kjeldahl flasks and likewise, 10 towers are needed. In all of these tests where carbon dioxide is collected the Kjeldahl flasks are swept out with air purified by passing through soda lime before starting the determination. Originally hydrogen was used to secure aeration, but in this work purified air has proved more convenient and perfectly satisfactory.

During the process, as suggested above, continuous and vigorous shaking is provided. The power is secured by attaching to an electric fan motor.

That thorough mixing is insured is evident from the fact that the flasks travel a path of 2 inches, with a back and forth somewhat rotary movement, about 400 times each minute. This gives 240,000 shakes in the 10-hour run. These figures are given merely to emphasize the difference between the modification presented here and an occasional shaking by hand. The 10-hour run is adopted partly for convenience and partly for the sake of accuracy.

With this method, as with the Truog and MacIntire methods, double titration with phenolphthalein and methyl orange is necessary. There is always a source of error in such a titration, but its magnitude seems insignificant in comparison with other variations. When a 20-gm. soil sample is used and the carbon dioxide titrated against N/10 acid, each cubic centimeter of the titration represents a lime requirement of 1000 pounds of calcium carbonate per 2,000,000 pounds of soil. In the results, a blank on both the soil and the alkali used for absorption is always subtracted. More accurate titrations can be made when the amount of carbon dioxide is not excessive. The end point is not distinct when the carbon dioxide evolved is the equivalent of more than about 20 cc. of N/10 acid. The amount of carbon dioxide may, of course, be regulated somewhat by diminishing the size of the soil sample in case there is too much for accurate titration.

### *Modifications*

In trying to improve the method a number of variations have been used. The solubility of calcium carbonate in water at 20° C. is about 1.2 parts per 100,000. In N/10 sodium chloride it is three times as great. If the solubility of the carbonate were the limiting factor in the reaction, then the use of a dilute sodium chloride solution instead of pure water should hasten the evolution of carbon dioxide. The carbon dioxide is less soluble in the presence of chlorides and, therefore, aeration should be facilitated also. The test, however, shows little effect on the final results. While a strong solution of chloride, has a depressing effect upon the solubility of lime, it would prevent any bacterial action which might liberate carbon dioxide. Süchting, in an attempt to improve the method, has suggested that there is a fermentative action which occurs, introducing an error in the determination, and it was thought at one time that this might be a factor to consider. But since a soil blank is run, such a provision seems superfluous. The blank on the soil is always small, seldom equivalent to more than 1 cc. of N/10 acid. It is also more or less constant in value for the different soils, and considering the results where antiseptic conditions have been provided it may be concluded that with a blank, the method is perfectly reliable, without any provision to reduce fermentations which might occur.

Calcium chloride, which was used in a similar way, has both the effect of increasing the solubility of lime with an accompanying facility of aeration, and

the common ion effect. Still another modification was the use of a few drops of toluene in the water. This should insure sterilization when long runs are being made and it seems also to have an effect of speeding up the reaction. The stimulation may be due to some chemical change brought about with the soil as has been suggested to occur in partial sterilization studies made by Pickering (5). Should any chemical change occur it would likely become a source of error rather than an advantage to the method.

A modification which should theoretically prove effective in hastening the reaction is the use of sodium nitrate solution. The sodium nitrate should liberate the soil acids in the same way as in the Hopkins method and the calcium carbonate which is present in large excess, should neutralize the acids as fast as liberated. The effect should be to permit the reaction to go to completion as though a number of extractions were made according to the original Hopkins plan. In actual practice the reaction is hastened, but it has not yet been considered of special value. The recovery should apparently be similar also, when chlorides of calcium and sodium are used, but the action has sometimes proved depressing rather than stimulating. These salts have a precipitating effect upon the soil colloids, as is observed by the clear supernatant liquid a short time after the machine has stopped shaking. An action of this kind might account for the retardation of the reaction, perhaps by rendering the soil acids more slowly soluble, and a consequent lower lime requirement is indicated. But the vigorous shaking has the reverse effect of breaking up the soil and apparently increasing the amount of colloidal material. It is only on very long runs that there is a marked indication of this kind, however.

The predominating factors with the method so far as studied seem to be the thoroughness of shaking and length of time of running. It has been found that scarcely any reaction occurs in several hours when the mixtures stand without shaking. It is found also that the evolution of carbon dioxide is a continuous process and probably never entirely ceases. In time, however, evolution is so slow that the amount evolved is little more than that ascribable to experimental error. This might seem to throw the method into disrepute, but these studies have led to a high valuation of the method. Below are given in more detail some comparisons and results.

#### *Water vs. saturated calcium chloride<sup>2</sup>*

In this determination 100 cc. of water was used, but a smaller amount has since been decided upon as adequate and more expeditious to the aeration.

The results in table 4 show the depressing effect of a concentrated calcium chloride solution upon the rate at which carbon dioxide is evolved. Though

<sup>2</sup> All results unless otherwise stated are expressed as pounds of  $\text{CaCO}_3$  per 2,000,000 pounds of soil.

the carbon dioxide is less soluble in concentrated chloride solution and should, therefore, aerate more rapidly than from pure water, the reaction is markedly retarded, probably on account of the insolubility of the carbonate in the concentrated calcium chloride. Evidently, too, the hydrochloric acid is not replaced to any extent from the calcium chloride by the soil acids, or else the reaction would have been speeded up. It is easily observed, also, that the evolution of carbon dioxide gradually decreases in rate, by far the greater part having come off during the first three hours.

*Dilute solutions of sodium and calcium chlorides*

There is little difference in the effect of dilute solutions of the chlorides of sodium and calcium, as shown in table 5.

TABLE 5

*Lime requirement by the Tacke method, comparing 5 per cent NaCl and 5 per cent CaCl<sub>2</sub>, and varying the time of running*

TREATMENT	LIME REQUIREMENT		
	5 hrs.	8 hrs.	21 hrs.
	lbs.	lbs.	lbs.
50 cc. 5 per cent NaCl and 2 gm. CaCO <sub>3</sub> .....	5,725	6,750	7,050
50 cc. 5 per cent CaCl <sub>2</sub> and 2 gm. CaCO <sub>3</sub> .....	5,660	6,480	7,020

Probably the dilute salts hasten the reaction somewhat but the effect is not marked, as is shown by comparing the requirement indicated here at the end of 8 hours with that shown at the end of 9 hours when pure water is used. These figures indicate again, as do all others, that the evolution of carbon dioxide has slowed up very much by the end of 8 hours.

*The effect of varying the amount of soil and carbonate used*

To determine the effect of varying the amount of soil, 50 gm. were used in comparison with a 20-gm. sample.

TABLE 6

*Lime requirement by the Tacke method, showing effect of varying the amount of soil used, with two periods of running*

AMOUNT OF SOIL	TREATMENT	LIME REQUIREMENT	
		9 hrs.	21 hrs.
gm.		lbs.	lbs.
20	50 cc. pure H <sub>2</sub> O and 2 gm. CaCO <sub>3</sub> .....	5,925	7,350
50	50 cc. pure H <sub>2</sub> O and 2 gm. CaCO <sub>3</sub> .....	5,790	7,300

The results are evidently not affected by the size of the soil sample so long as it is not too large to prevent intimate mixing with the carbonate and aeration is not depressed. It will be observed that there is some variation in corresponding runs from day to day. This is due partly to variations in sampling the soil, to humidity conditions and other factors which cannot be controlled. An air-dry soil must take up considerably more water in a saturated atmosphere than in one that is relatively dry, and consequently not the same actual weight of soil is used in all tests. These errors, however, are not significant for the purposes of the tests made and the method is no more susceptible than others in these respects.

The amount of carbonate also was varied, 5 gm. being used instead of 2 gm., and this proved likewise to be without influence on the determination.

In this test 10 per cent calcium chloride solutions were used, but comparative results could not have been different with pure water. The only essential, therefore, is that there shall be an excess of carbonate and sufficient water to insure intimate mixing. A large amount of water retards aeration very decidedly, as was found in standardizing a bicarbonate solution by this method of decomposing carbonates.

TABLE 7

*Lime requirement by the Tacke method showing the effect of varying the amount of  $\text{CaCO}_3$  used in a dilute  $\text{CaCl}_2$  solution*

TREATMENT	LIME REQUIREMENT
	<i>lbs.</i>
50 cc. 10 per cent $\text{CaCl}_2$ and 2 gm. $\text{CaCO}_3$ .....	6,825
50 cc. 10 per cent $\text{CaCl}_2$ and 5 gm. $\text{CaCO}_3$ .....	6,700

Period of running, 9 hours. Amount of soil used, 20 gm.

Incidentally, we may observe here the speeding-up of effect a dilute solution of the chloride of calcium. When this result is compared with the requirement indicated by pure water at the end of 9 hours, a difference of about 1000 pounds per acre is observed. Any method becomes more consistent, however, with familiarity and standardization, and it is quite probable that some of the variations could be reduced if all operations were carried out under a rigidly-controlled procedure. Therefore, it is not desirable to over-emphasize apparent effects of different treatments.

#### *Effect of toluene*

It should be understood that only a few cubic centimeters of toluene were added to pure water in this test, usually about 2 cc. This is too small an amount to have any marked effect upon the solubility of either the carbonate or the carbon dioxide evolved. The results are given in table 8.

TABLE 8

*Lime requirement by the Tacke method, showing the effect of toluene compared with pure water and  $\text{CaCl}_2$  solution*

TREATMENT	LIME REQUIREMENT		
	9 hrs.	24 hrs.	36 hrs.
	lbs.	lbs.	lbs.
50 cc. $\text{H}_2\text{O}$ , 2 gm. $\text{CaCO}_3$ and toluene.....	6,600	7,200	8,400
50 cc. $\text{CaCl}_2$ and 2 gm. $\text{CaCO}_3$ .....		7,175	8,200
50 cc. pure $\text{H}_2\text{O}$ and 2 gm. $\text{CaCO}_3$ .....	6,200		

The toluene seems to have somewhat stimulating action on the rate of evolution of carbon dioxide during the first 9 hours. Where the vacancies are shown, the determinations were not made. In general, it may be said that none of the compounds tried have been of value to the procedure, and their use for routine work has been rejected.

#### *Results with gray silt loam*

A few determinations were made with the second soil which shows the same general behavior. In laboratory work the method has been used with still other soils and so far as tested, it works equally well with all.

TABLE 9

*Lime requirement by the Tacke method showing the effect of toluene compared with water and  $\text{CaCl}_2$  on the gray silt loam soil*

TREATMENT	LIME REQUIREMENT		
	9 hrs.	24 hrs.	36 hrs.
	lbs.	lbs.	lbs.
50 cc. $\text{H}_2\text{O}$ , 2 gm. $\text{CaCO}_3$ and toluene.....	4,400	5,800	
50 cc. 10 per cent $\text{CaCl}_2$ and 2 gm. $\text{CaCO}_3$ .....	4,200	5,600	
50 cc. $\text{CaCl}_2$ and 2 gm. $\text{CaCO}_3$ .....	4,425	5,700	6,700

The only reason for using toluene in the water with this soil is that the run happened to be made when the effects of toluene were being tried in comparison with calcium chloride solutions. The water alone could not have acted much differently. It is observed that there is considerable carbon dioxide evolved after the first 9 hours' run. This would indicate that either the aeration was too slow to permit the reaction to go forward rapidly, or that the acids of the soil were inactive. Both factors may have operated in this case, and the results emphasize the importance of maintaining standard and control conditions to allow the reaction to occur at the maximum rate.

*Discussion of factors influencing results*

*Temperature.* There are possible factors influencing the operation of the method, for which no accurate means of measuring the effect is available. Temperature increase means an increase in the rate of most chemical reactions, but it also means a decrease in the solubility of the carbonate, and there is no means of measuring the net effect of such variations. Even in the laboratory, if winter and summer extremes are compared, there must be large temperature ranges. This is one argument for continuing the shaking several hours. The long run tends to overcome momentary effects of temperature and other fluctuations.

*Rate of aeration.* A factor which is more important is the rate at which air is drawn through to carry over the evolved carbon dioxide. Though the stream of air may be regulated by means of glass stop-cocks of approximately the same bore and the size and rate of bubbles produced in the aeration serve as a guide, nevertheless, there is no quantitative check on the process. In general a rapid aeration has proved best, and a fairly rapid bubbling is perfectly safe from loss of carbon dioxide. In trials made, carbon dioxide has never been carried over into a second absorption tower. With a very large quantity of carbon dioxide, as might occur when residual carbonates are determined with this apparatus, there might be trouble and under such conditions it would be advisable to aerate slowly at first to insure complete absorption, and then after the first large evolution of gas has passed, to aerate more rapidly. At the end of the run it is especially advisable to speed up to secure as nearly complete aeration of the carbon dioxide as possible.

*Pressure of carbon dioxide.* Another factor which may influence the rate of reaction is the partial pressure of carbon dioxide in the Kjeldahl flasks where the reaction is occurring. Calcium bicarbonate is more than thirty times as soluble in water which is saturated with carbon dioxide as it is in pure water. Though saturation would never exist, the amount of carbon dioxide present at times is doubtless great enough to produce some effect. An excess of carbon dioxide would operate in another way, tending to retard the reaction according to the mass law. Here again the net effect is doubtful.

*Nature of soil acids.* Still another very important factor is the kind and strength of the acids occurring in the soil. The rate of reaction of calcium carbonate with soils must vary in proportion to the activity of the acids present, as previously suggested. It is quite evident that if this is true, the contact of soil and carbonate must be continued until the inequality in rate of reaction is overcome by the long-time run, or until there is a somewhat constant evolution of carbon dioxide in both cases. If run a short time only, one soil should show a much greater acidity than the other, while at the end of a sufficient time there should be little difference. For these reasons, it is believed that an extended time of shaking and aeration, tends to minimize a number of sources of error.



The rate at which carbon dioxide is evolved may be partially accounted for if the soil itself, not its solution, is highly "buffered," as would be manifested in a marked tendency of the soil mass to resist change in reaction with the addition of a base. To show buffer action it is necessary only that there be present a weak acid and the salt of the weak acid, or in the case of alkalinity a base and salt of similar nature. Without question many soils present ideal conditions for such a buffer action. This is indicated also by the fact that it is seldom possible to obtain soil acids in a water extraction. Insolubility alone would, of course, account for this phenomenon were it not for the fact that some soluble acids must be present and would be extracted were they not buffered. A logical way of accounting for their inactivity is found in this explanation. A buffer effect may account, therefore, for the large reserve acidity shown by some soils. Though the methods may indicate a large lime requirement the hydrogen-ion concentration is small. This may explain also the activity of microorganisms in very acid soils. The acidity reported is mostly reserve acidity and the organisms, for example the nitrifiers, are

TABLE 10

*Lime requirements by the Tacke method, showing a comparison of the effect of normal nitrate solution with that of pure water*

TREATMENT	LIME REQUIREMENT	
	3 hrs.	10 hrs.
	lbs.	lbs.
50 cc. pure H <sub>2</sub> O, and 2 gm. CaCO <sub>3</sub> .....	6,000	6,600
50 cc. N/1 NaNO <sub>3</sub> and 2 gm. CaCO <sub>3</sub> .....	6,800	

active because the harmful acidity or hydrogen-ion concentration is below that concentration which is inimical to their life processes. Though the Tacke procedure does not measure the hydrogen-ion concentration accurately, it does measure the rate of reaction between carbonate and soil acids and, therefore, indicates the degree of activity and probable harmfulness of the acidity. This, rather than total acidity, is really the important point in a determination, and unless a method indicates something in regard to the activity of the acids it is not of the greatest practical value.

That much of the soil acidity is potential rather than active is indicated by some further results. Hopkins believes as suggested above that repeated extractions of the soil, taking the sum of all the fractions thus obtained, will give the total acidity. The data given in table 10 with normal sodium nitrate are notable.

In the 10-hour run with pure water the soil used shows an acidity of 6600 pounds, or 200 pounds less than the amount obtained in the 3-hour run with sodium nitrate. Since this investigation has been only started, it is not yet

possible to draw any conclusions. But it should be expected that many of the soil acids would be too weak to displace a strong acid, such as nitric, from its salt. However, since calcium carbonate is present to neutralize the acid as fast as liberated and since, too, the carbonate itself reacts directly with the inactive soil acids, the use of sodium nitrate might facilitate the activity of what are sometimes called "latent" acids. The nitrate increases the solubility of the carbonate very decidedly also, since calcium nitrate is soluble to the extent of one part in two of water. Any effect of this kind must tend to hasten the reaction.

More evidence is found in the test recorded in table 11, that carbon dioxide practically never ceases to be evolved.

It is easily observed that the rate of evolution of gas gradually decreases, and it seems probable that it should have finally a more or less constant value. The proper place to stop becomes, therefore, the important question. With former workers, the constant evolution of carbon dioxide has been the big objection to the method. Nevertheless, there are some good reasons for confidence in the method. The constant evolution of carbon dioxide means only that the same thing is occurring as takes place in the field. Namely,

TABLE 11

*Lime requirement by the Tacke method, showing continuous evolution of CO<sub>2</sub>*

TREATMENT	LIME REQUIREMENT						
	9 hrs.	24 hrs.	36 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
50 cc. CaCl <sub>2</sub> and 2 gm. CaCO <sub>3</sub> ...	6,700	7,175	8,200	8,950	9,275	9,575	10,075

that as fast as the acids present are neutralized, more develop, the rate of development being perhaps infinitely slow normally, but under the conditions of the test speeded up to a measurable rate.

This is doubtless partly due to buffering effects. In the field, too, there is the leaching of bases and the assimilation of bases by plants. Probably however, most plants use nearly as much of the acid radical. At least the production of crops must tend toward the preservation of bases, since the decay of plant roots and other residues leaves the basic radical in the soil, while the organic acid radical is completely oxidized under normal conditions. And when a base is leached there is removed with it an equivalent amount of acid in the form of its salt. Only, therefore, when a bicarbonate is leached is a functioning base removed, and increase in active soil acidity is not primarily the result of leaching of bases but rather the result of the development of new acids in the absence of base. So long as a base is in combination with an active acid it is useless for further neutralizing action. The continual leaching of salts, however, must finally result in a base-poor soil and lack of basic elements

for plant growth. Then when new acids develop, plant growth is still further retarded until the acidity is neutralized by application of lime. But the Tacke method is no less accurate in measuring the existing acidity whatever the rate or manner of its accumulation in the soil.

It is found, in unpublished results, that where lime is applied in large excess of the acidity, the method always indicates a lime requirement. This is apparently a correct indication. In the soil under field or greenhouse conditions, only the rather active acidity is neutralized because the base is not in sufficiently intimate contact with the soil. It is fairly certain that acid soils have local neutral or alkaline areas. It is just as logical that there must be local acid areas in any soil even though limed. In the operation of this method in the laboratory, however, with the vigorous shaking and constant aeration, more complete neutralization is secured. This, with the hydrolytic reactions that doubtless occur, gives a greater evolution of carbon dioxide than is actually represented by harmful acidity. In the field several years may be required for a similar action. It is known, however, that when soils are limed to the supposedly neutral point, they sometimes become acid again very quickly. Some experiment stations, therefore, always recommend an application of from one to three tons in excess of the indicated lime requirement. When methods differ so widely in their indications, lime requirement really means little unless both the method and type of soil are designated.

In this work some further data have been secured illustrative of the indefiniteness of the end point in the reaction between lime and acid soil. For a qualitative indication, the method originated by Truog has been used. This procedure, sometimes spoken of as the lead acetate method, has been investigated at the Ohio Station (1) and found as reliable as any qualitative test, so that it seems safe to trust its indication of the neutral point. The neutral point with the lead acetate test corresponds nearly exactly with the point where a constant acidity is shown by the Tacke method, running 10 hours. This is on acid soils (results unpublished) which have been limed at rates increasing successively by one ton, so that the soil acid equivalent in lime must occur somewhere in the series. It is evident, therefore, that the method goes beyond the point at which hydrogen-ions are liberated in sufficient quantity to free hydrogen sulfide from zinc sulfide, to an extent such that lead acetate paper is darkened. This excess or constant acidity is somewhere about 10 to 15 hundred pounds per acre, depending, of course, somewhat upon the length of time since liming the soil. These results indicate that a run for a shorter time on the soil used would be adequate. Possibly half as long might be sufficient. But the constant or excess requirement introduces no error in the studies, since its value is known and the excess is the same for all treatments within experimental error. To run a little in excess of the true lime requirement is only working on the side of safety. It might be argued, too, that this potential acidity should be provided for, since it may eventually

develop to a harmful extent in the field. For experiment station use, however, a run of from 5 to 7 hours would seem to give accurate and comparable results, the exact time depending upon the soil and somewhat upon convenience. The time of running is evidently largely susceptible to adaptation to routine work, and this without introducing any serious error.

### *Conclusions*

The possibilities of the Tacke method are very encouraging though there are some weak points. Since there is only pure water, pure calcium carbonate and soil, with the accompanying reaction products, present in the equilibrium systems, the results should not be extreme in either deficit or excess, and probably they may be about correct quantitatively as well as accurate qualitatively.

If time permits it is hoped that further developments may be made to determine more exactly, if possible, the end point to consider in the reaction. It may be possible by the use of colored indicators and known hydrogen-ion concentration to follow the reaction in the decomposition of pure carbonate. By comparing this reaction rate with that of the soil, a more definite conclusion may be derived in regard to the length of time for conducting the test. Such studies should also give further knowledge in regard to the nature of soil acids, their activity, degree of buffering and other disputed points.

### SUMMARY

#### *Part I*

1. Methods which depend upon the liberation of an acid from its salt do not give total acidity and indicate a lime requirement which depends both upon the soil and salt used.
2. Methods employing heat or a strong base are not reliable since their indications are likely to be both excessive and inconsistent.
3. The nature of the soil acids is a very important factor which must be considered in studies of lime requirement.
4. The Tacke method has proved the most satisfactory of any method tried.

#### *Part II*

From a study of the Tacke method of determining the lime requirement of soils, the following conclusions are indicated:

1. Pure water is a reliable medium for bringing about the reaction between the acid soil and carbonate.
2. The use of dilute solutions of calcium or sodium chloride hastens the reaction only to a limited extent.
3. A concentrated solution of the above salts may prevent fermentative reactions, but such a provision has proved unnecessary. The rate of reaction is somewhat depressed by concentrated chlorides.

4. Toluene has proved of no value to the method. An antiseptic is evidently not needed.

5. The use of normal sodium nitrate hastens the reaction but its value is not yet established.

6. The time of running, the rate of aeration and the vigor of shaking are the most important factors in the Tacke method.

7. The rate of aeration should be maintained at a maximum.

8. The effects of temperature and the partial pressure of carbon dioxide cannot be determined.

9. A long run, 5 to 10 hours, adds to the reliability of the method, tending to overcome many momentary influences.

10. The activity of soil acids varies greatly as measured by the rate of evolution of carbon dioxide. The more reactive acids react at once, the less reactive only after long contact and thorough mixing of soil and carbonate, and more complete removal of carbon dioxide liberated.

11. The method is not only consistent in indicating total acidity but in a limited way measures the toxicity of the soil acids.

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# REPORT ON THE EXAMINATION OF COMMERCIAL CULTURES OF LEGUME-INFECTING BACTERIA

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The object of this work was to test the efficiency, purity and identity of commercial cultures of nitrogen-gathering bacteria. Since the time of Caron (2) who first proposed inoculation of the soil with beneficial non-symbiotic nitrogen-gathering bacteria, and of Nobbe and Hiltner (4) who first used the method of seed inoculation, a great many cultures have been placed upon the market. Repeated failures with non-symbiotic nitrogen-gathering bacteria have led to a very limited use of such cultures at the present time. However, one firm's product (Alphano Humus Co.), which has been tested repeatedly by the author contains vast numbers of *Azotobacter*.

Greater by far is the importance of the symbiotic nitrogen-gatherers. With the discovery of the functions of nodules on the roots of legumes by Hellriegel (6) in 1886, a new epoch was begun in the science of agriculture. His announcement that the organisms within the nodules are able to assimilate elemental gaseous nitrogen from the atmosphere, has been verified so many times by scientists of high repute that no doubt exists today on this question.

Immediately after this discovery, men began to commercialize these nitrogen-gathering bacteria. Thus we find the work of Salfeld (8) and Schmitter (9) in Germany demonstrating the value of inoculating moor soils with soils infected with the nitrogen-gathering organisms, especially for the growth of legumes. Later on "Nitragin," a German product, appeared on the market. Contradictory results were obtained from its use, but the commercialization of the nitrogen-gathering bacteria gradually became well established and other preparations were manufactured and sold to farmers for the purpose of inoculating legumes. Harrison and Barlow, of the Ontario Agricultural College, did pioneer work in North America and originated the method of growing *Bacillus radicicola* on a nitrogen-free medium. This was soon copied by others until, at the present time, most of the cultures on the market are pure cultures of definite varieties of the nitrogen-gathering bacillus, grown on a nitrogen-free or, rather, a nitrogen-poor agar medium.

Recently, however, more attention has been paid to the distribution of *B. radicicola* in nature's own medium, i.e., the soil. Muck which has been specially treated is also being used to some extent. Liquid cultures have appeared

to be shorter-lived than the cultures on solid media, and hence their use is somewhat limited at the present time. The United States Department of Agriculture still makes use of liquid cultures.

#### TESTING COMMERCIAL CULTURES

Little has been done on the methods of testing cultures of legume bacteria. Garman and Didlake (3) of the Kentucky Agricultural Experiment Station have developed the growing of the plants in sterile tubes of agar and noting nodule formation. Temple (10) of the Georgia Agricultural Experiment Station tested a considerable number of commercial cultures of *B. radiculicola* for various legumes in 1916. He made bacteriological counts of the bacteria in each culture and carried on some vegetation experiments in the laboratory and field, as well. Most of the cultures tested by him were satisfactory but there were some exceptions. As early as 1905, Harding and Prucha (5) showed that the "Cotton cultures" distributed by the United States Department of Agriculture were unreliable. These investigators are among the first to have tested commercial cultures of legume bacteria.

So many unsuccessful results were obtained by New Jersey farmers during the past two seasons, following the use of certain commercial cultures of nitrogen-gathering bacteria, that the New Jersey Agricultural Experiment Station deemed the matter important enough to deserve some study. Accordingly, plans were devised to obtain samples of the more common cultures on the market, from the county agents and from the farmers themselves. These samples were sent to the laboratories of the Experiment Station and tested.

#### METHOD USED

Since no official procedure exists for the examination of such cultures of *B. radiculicola*, suitable methods were devised for this work. These are briefly as follows:

The medium used was a slight modification of Ashby's medium and was composed of mannite, 12 gm., mono-potassium phosphate 2 gm.; magnesium sulfate 0.2 gm., sodium chloride 0.2 gm., calcium sulfate 0.1 gm. and calcium carbonate 1 gm. These salts were added to 1000 cc. of distilled water and 15 gm. of purified agar. For general plating of all varieties of *B. radiculicola*, this was found to be equal, if not superior, to several other media with which it was compared. Among those tested were Temple's saccharose agar, Harrison's wood ash agar, Fred's medium no. 2 and Ashby's medium, where 12 gm. of dextrose was substituted for the mannite. Each particular group of *B. radiculicola* appears to prefer a certain medium, hence the conclusion was reached that no single medium is entirely satisfactory for the plating of different varieties of this organism. In order to obtain comparable results, however, the same medium should be used for plating out different cultures of legume bacteria.

The medium used was made up in 5-liter batches, tubed and kept in an ice box until used, to avoid evaporation and consequent deterioration. The medium was always sterilized at a pressure of 1 atmosphere and later melted by placing in flowing steam.

### *Method for agar cultures*

*Plate method.* Twenty cubic centimeters of sterile tap water was carefully pipetted into the bottle of material to be tested, the stopper inserted and violently agitated for 15 minutes in an electric soil shaker. Then portions of the liquid were taken by means of pipettes and transferred to 250-cc. Erlenmeyer flasks, containing 90 or 99 cc. of sterile tap water, according to whether a 10-cc. or a 1-cc. portion was used in the dilution. From this flask, other dilutions were made. Each dilution flask or tube was shaken violently for 1 minute; this was found to necessitate the use of rubber or ground glass stoppers. Basing calculations upon the contents of the sample, plus the 20 cc. of sterile water added to it, the usual dilutions made were 1: 100, 1: 10,000 and 1: 100,000; although in exceptional cases dilutions of 1: 10 and 1: 1,000,000 also were used.

Plates were made in duplicate for each dilution, and were incubated at a temperature of 25°C. for 10 days, when the colonies were counted. It was observed that in the case of some cultures, especially soybean and cowpea bacteria, a longer incubation period was sometimes necessary in order to count the colonies macroscopically. In one case, it was only after 20 days of incubation that colonies of *B. radiculicola* (soybean variety) were visible to the unaided eye. On the other hand, the alfalfa and vetch bacteria sometimes formed moderately large colonies in 3 days, and had spread so much as to render counting difficult after 10 days.

### *To test nodule production on the host plant*

For this work the previously sterilized seeds of the host plants were planted in tumblers of sand to which had been added sufficient plant-food for normal growth. The first attempt was a failure, due to the fact that the concentration of the salts was so great as to hinder good root development, and hence only a few nodules were produced. A very low concentration of salts without nitrogen seems to work best for most legumes. The sand was made up to the optimum moisture content with distilled water and covered with Petri dishes until the plants had germinated well. Nodule counts were usually made after 30 days.

*Agar tube method.* Large tubes containing about 250 cc. were filled two-thirds full of Ashby's medium. The amount of agar added was 7.5 gm. per liter instead of 15 gm. After sterilization and cooling, sterilized seeds of the legumes which were being tested were placed aseptically in these tubes and allowed to grow. The medium was translucent and allowed a good view of



any nodules which developed. Of course, previous to planting, the sterilized seeds were inoculated with the culture of legume bacteria under test, except in the check tubes. For the purposes of sterilizing the seeds, the latter were well washed in sterile water, and then in  $\text{HgCl}_2$  of a strength 1:500 for 2 minutes. They were then very thoroughly washed in many changes of distilled water. The question arose in the writer's mind as to whether the  $\text{HgCl}_2$  could be all washed away; hence, several beans which had been treated as above were crushed and extracted with hot water and filtered. On testing this extract for mercury only a trace was found; yet even this might be toxic to *B. radiculicola* to a greater or less degree.

### *Testing liquid cultures*

Exactly the same methods were used in testing liquid cultures as was employed with the agar cultures just described. The only exception was that no sterile water was added to the sample previous to shaking and plating.

### *Testing soil and muck cultures*

Many difficulties were encountered in making counts of soil and muck cultures because they were usually not pure cultures and contained many species of fungi, yeasts, Actinomyces and foreign bacteria. These samples were usually guaranteed to inoculate all legumes and, therefore, contained many varieties of *B. radiculicola*, which are indistinguishable from one another on plates. A fair estimate of the total number of *B. radiculicola* present may be obtained by the plate method, and after one becomes sufficiently expert, he may also be able to differentiate between certain dissimilar varieties of this organism, as for example, the alfalfa type from the soybean type; but for accurate diagnosis of the samples, the plate method is not to be recommended. It does give a good idea of other organisms which may be present, e.g. *Azotobacter*, Actinomyces and yeasts, many of which grow luxuriantly upon Ashby's medium. The procedure used for plating soil or muck cultures is the same as that mentioned for agar except that weighed quantities of soil are used to make the dilutions. It was noticed that as a general rule, the colonies developing upon plates poured from soil or muck were usually smaller, developed more slowly, and were less characteristic than those developing from pure cultures on agar in solution. This may be only a result of the presence of other organisms on the plates, but seemed to be true in most cases. The so-called association between *B. radiculicola* var. soybean and *Azotobacter chroococcum* was also very evident on many of the plates. Where *B. radiculicola* colonies were present in large numbers the *Azotobacter* colonies soon spread over large areas of the plate. This fact has been previously commented upon by Manns (7) of the Delaware Agricultural Experiment Station. Some species of *Penicillium* are also helpful to the growth of *B. radiculicola* colonies, and in several instances the writer has observed a growth of this bacterium entirely covering

the fungus mycelium. Recently the late Dr. Burrill (1) of the Illinois Agricultural Experimental Station has reported similar observations.

To test nodule production on the host plant, the same procedure was followed as is given under agar cultures. The seeds were inoculated rather than the soil in every case. Agar tube cultures were usually a failure where soil or muck was employed as the inoculum. This was because of the fungi and proteolytic bacteria present which decomposed the highly nitrogenous legume seeds before they could germinate.

As it is known that cultures of legume bacteria deteriorate after some time, the cultures were examined within a few days of the time they were received in the laboratory. In some cases, the tests were repeated after a month or more upon the same samples, to see how long the bacteria remain viable in the sample bottle.

The results are given in table 1.

#### DISCUSSION OF RESULTS

Of the official samples only two were classed as wholly poor, but six were partly poor, i.e., some cultures which were claimed to infect all legumes, failed to do so when tested. A much greater proportion of the unofficial samples were poor. This was probably due to several reasons. Several of the companies were notified that their products were not satisfactory, hence it is probable that the standard was raised during the spring and summer of 1917. Also, a few of the samples were allowed to remain in the laboratory for some time before testing and it is seen from the data that such cultures were usually poor.

More samples of the soybean organisms were condemned than of other varieties. This is probably due to the scanty and slow growth which this type of *B. radiculicola* makes on artificial media. Soybeans are also harder to inoculate than most seeds.

The high standard of purity of the cultures was gratifying. Only a few which claimed to be pure did not live up to the guarantee. It is a question in the writer's mind whether a pure culture is better than an impure one, especially in soil or muck cultures. Of course, in agar and in liquid cultures proteolytic bacteria and fungi should not be present, but certain species of *Penicillium* and *Azotobacter* actually aid in the growth of *B. radiculicola* on media, and probably in the soil.

The price per acre lot asked for the cultures varied from fifty cents for the Standard Nitrate Agencies Product to two dollars for the Earp-Thomas Farmogerm. Little correlation was noticed between the price of cultures and their efficiency. Most of the samples tested were agar cultures, but the use of soil and peat is rapidly coming to the fore and in the not distant future, will probably entirely displace the liquid and agar cultures. There are several reasons for this. Temple (10), Manns and Goheen (7) and others have demon-

strated that *B. radiculicola* lives and multiplies in suitable soils for long periods of time, if optimum conditions are observed. In culture solutions it soon dies out after a few months, and even a number of agar cultures stored unopened in the laboratory for a few months, showed great reduction in numbers.

1. The soil or muck cultures possess greater viability than agar or liquid cultures.

2. More bacteria are usually present in an acre size of soil or muck culture than in agar or liquid cultures. Greater bulk is also used, thereby insuring better inoculation.

3. There is better aeration and the medium is a more natural one than that of agar or liquid cultures.

4. Many types of *B. radiculicola* may be added to the same culture of soil or muck; this obviates the need of using a different culture for each legume.

5. Other types of *B. radiculicola* are introduced into the field, if the culture happens to be a composite one. This minimizes the need for inoculation in after years if other legumes are to be grown.

6. Sterilization of the soil or peat is not always necessary or desirable, since some organisms present in soil aid the development of *B. radiculicola*.

7. Heat, light and exposure do not affect soil cultures as much as agar or liquid cultures. Even after soil becomes air-dry, many of the bacteria may live in the film of hygroscopic moisture surrounding each soil particle. If muck is used the water content is so high that even if it does dry out considerably it will still contain sufficient moisture for bacterial growth.

8. The ease of inoculating with soil cultures should give them preference over liquid cultures. Whatever soil is left over from inoculating the seeds may be mixed with other soil and drilled with the fertilizer, thus insuring better inoculation of the land.

9. The price is about the same for both soil and liquid cultures, but the cost of manufacturing the former is probably less than that of the latter.

The number of bacteria per cubic centimeter in the various cultures varied greatly. No definite minimum limit to the number of bacteria present in a culture can be safely set, for obvious reasons. The infecting ability of 500,000 organisms in one culture may be equal to that of 500,000,000 of another; in fact, some of the vegetation experiments carried on in the laboratory pointed to this very thing. But few cultures containing less than 1,000,000 cells per cubic centimeter were effective. The number of bacteria in an acre-size sample is a better means of comparison. The usual range encountered for average samples was from 100,000,000 to 1,500,000,000 but some samples gave higher and some lower counts.

The efficiency of a culture to infect the host plant is the only suitable test for determining its agricultural value. Of course, a good indication is obtained in the plate counts, and if the numbers are high, the culture is usually good. No culture which gave low plate counts was classed as satisfactory when the

vegetation tests were completed. It was noticed that where only a few nodules were found on the roots, they were usually of a larger size than where the infection was more general. To be classed as "good" a culture, when applied according to directions should produce several nodules per plant. When less than one nodule per plant was produced, the culture was classed as "poor."

Most of the samples had no time guarantee, hence the farmer has no means of knowing whether the cultures are old or fresh. Control tests conducted by the State Agricultural Experiment Station seem to be the only effective method of guarding the farmers against unscrupulous or careless dealers.

In this connection it is important to know the number of seeds of the common legumes which are planted to the acre. This gives us an approximation of the relative number of bacteria required for different crops. Some seeds are large, others small, some are smooth while still others are rough. These are factors which enter into the ease of seed inoculation. The relative difficulty of inoculating soybean seed may be due to the smooth oily seed coat to which the bacteria do not readily adhere. Some figures are given below regarding the number of seeds of our common legumes usually planted per acre.

Alfalfa (20 pounds).....	5,100,000
Sweet clover (20 pounds).....	4,800,000
Red clover (12 pounds).....	3,300,000
Alsike clover (12 pounds).....	8,400,000
White clover (10 pounds).....	8,000,000
Crimson clover (12 pounds).....	1,500,000
Lespedeza (20 pounds).....	7,400,000
Spring vetch (60 pounds).....	540,000
Winter vetch (60 pounds).....	960,000
Soybean (60 pounds).....	150,000
Field bean (80 pounds).....	120,000
Cowpea (70 pounds).....	250,000
Field pea (120 pounds).....	265,000
Peanut (30 pounds).....	36,000
Velvet bean (12 pounds).....	18,000
Beggar weed (10 pounds).....	4,200,000

These data show that, broadly speaking, the cultures tested contained sufficient bacteria to allow for several cells per seed. In many cases, the bacteria were present in sufficient numbers to allow 100 or even more per seed. The number present should probably be larger for small-seeded legumes, like the clovers and alfalfa, than for cowpeas or field beans. The minimum number of cells per seed required to inoculate any legume thoroughly has not been worked out, and wide variations will surely occur from any standard that may be made.

## PRACTICAL DEDUCTIONS FROM THE TESTS

1. Only two cultures were classed as "poor" and four as "partly poor." Of these latter, two were "good" in all tests except those with the pea bean, a rare legume.

2. The purity and general condition of the cultures examined were, generally speaking, very good.

3. Soybeans seem to be harder to inoculate than most of the common legumes. Many of the cultures failed to give satisfactory results with this plant. The soil-transfer method is recommended for soybean inoculations except when the commercial cultures are known to be of good quality.

4. Soil or muck cultures are excellent carriers of legume bacteria.

5. The plate method of testing pure cultures gives a good indication of the infecting ability of the organisms. This test must be verified by growing the plants themselves and examining the roots for nodules.

6. A standard for the lower limit of "bacteria per acre-size sample" cannot be set until much more work has been done on this subject, and probably not at all because of the variability in the physiological efficiency of the organisms themselves.

7. Control tests on commercial cultures of legume bacteria should be made by the State Agricultural Experiment Stations for the benefit of the farmer.

## UNOFFICIAL TESTS

Besides the foregoing official samples, about 20 unofficial samples were tested for the extension department of the Experiment Station and others. Many of these samples were obtained directly from the manufacturers, while some were brought from various seed houses or obtained from farmers. The results obtained are briefly stated in table 2. It should be said that the following samples were procured and tested during the fall of 1916 and the spring of 1917.

## ACKNOWLEDGMENT

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TABLE 1  
\* Tests of official samples of commercial legume cultures

NAME OF CULTURE	LABORATORY NO.	DATE RECEIVED	SIZE	PRICE	MEDIUM USED	VARIETY OF LEGUME	BACTERIA PER CENTIMETER*	BACTERIA PER ACRE	PURITY	EFFICIENCY IN NODULE PRODUCTION	REMARKS
Farnogerm—Earp-Thomas Farnogerm Co., Bloomfield, N. J.	1	July 27, 1917	1 acre	\$1.50	White agar	Alfalfa	120 M†	3,600 M	Pure culture	Very good in both soil and agar	The bottle was aerated
Farnogerm—Earp-Thomas Farnogerm Co., Bloomfield, N. J.	2	July 27, 1917	1 acre	1.50	Muck	All crops	Total colonies 400,000	1,340 M	Not pure—180 M Actinomyces and 2 M fungi per gram	Alfalfa, clover, vetch—Fair. Soybeans, cowpeas, garden beans—Poor	Moldy smelling, no nodules on soybeans
Alphano Inoculant — Alphano Humus Co., New York City	3	July 27, 1917	Small sample		Muck	All legumes	3 M total B. radicicola		Not pure—4 M Actinomyces 110,000 <i>Asolobacter</i> per gram	Alfalfa, clover vetch, cowpeas—Good. Soybeans—Poor	Finely granulated Muck. Lupines Fair. Pea bean—None
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	4	July 27, 1917	½ acre	.50	Dark agar	Vetch	680,000	100 M	Pure	Fair	Bottle not aerated. Medium colored black with some substance
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	5	July 27, 1917	½ acre	.50	Dark agar	Crimson clover	980,000	1,274 M	Pure	Good	Bottle not aerated. Agar colored black
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	6	July 27, 1917	½ acre	.50	White agar	Alfalfa	1.37 M	164.4 M	Pure	Good	Agar not colored
Alphano Inoculant — Alphano Humus Co., New York City	7	July 25, 1917	1 acre	1.00	Muck	All legumes	1.37 M	22,330 M	Not pure 120,000 <i>Asolobacter</i> and 2.4 M Actinomyces per gram	Alfalfa, clover, cowpeas—Good. Soybeans and vetch—Fair. Pea bean—Poor	Finely granulated muck. Lupines—Fair

8	Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J. (From Virginia)	July 30, 1917	1 acre	\$2.00	White agar	Vetches	4.9 M	147 M	Pure	Good	Aerated container
9	Farmogerm—Earp-Thomas Farmogerm Co., Bloomfield, N. J.	July 30, 1917	1 acre	2.00	Light agar	Crimson clover	4.2 M	126 M	Pure	Good	Aerated container
10	Farmogerm—McElroy Shepherd Co., Bloomfield, N. J.	July 30, 1917	1 acre	1.50	Light-colored agar	Alfalfa	11 M	330 M	Pure	Very good	Aerated container
11	Nitrogem—H. K. Mulford Co., Philadelphia, Pa.	August 16, 1917	$\frac{1}{2}$ acre	.50	Light-colored agar	Cowpeas	160 M	19,200 M	Not pure. Fungi and Actinomycetes present	Good	No aeration
12	Alphano Inoculant — Alphano Humus Co., New York City	August 20, 1917	10 acre	8.00	Muck	All legumes	Total 2.1 M	36,000 M	Not pure. Many fungi, actinomycetes and other bacteria	All legumes except soybeans—Good. Soybeans—Fair	Finely granulated muck. Earthy odor
13	Alphano Inoculant — Alphano Humus Co., New York City	August 28, 1917	1 acre	1.00	Muck	All legumes	1 M	18,000 M	Not pure. Fungi <i>Asiobacter</i> and actinomycetes present	All legumes—Fair except soybeans—Poor	Finely granulated muck
14	Standard Seed and Soil Inoculation Co. Distributed by Bokhara Seed Co., Falmouth, Ky.	August 17, 1917	1 acre	.50	Agar	Vetch	11 M	330 M	Pure	Good	Container aerated
15	Standard Seed and Soil Inoculation Co., Troy, N. Y.	August 17, 1918	$\frac{1}{2}$ acre	.50	Agar	Sweet clover	30 M	1,800 M	Pure	Very good	Aerated container†

\* 20 cc. sterile water added to each sample.

† M., millions.



TABLE 1—(Continued)

NAME OF CULTURE	LABORATORY NO.	DATE RECEIVED	SIZE	PRICE	MEDIUM USED	VARIETY OF LEGUME	BACTERIA PER CUBIC CENTIMETER*	BACTERIA PER ACRE	PURITY	EFFICIENCY IN NODULE PRODUCTION	REMARKS
Farmogerm—Earl-Thomas Farmogerm Co., Bloomfield, N. J.	16	August 18, 1917	Garden size $\frac{1}{2}$ acre	\$0.50	Agar	Soybeans	1.5 M	180 M	Pure	Poor	No aeration. Putrid odor in sample on opening
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	17	August 20, 1917	$\frac{1}{2}$ acre	.50	White agar	Alfalfa	1.1 M	133 M	Not pure. A few fungi present	Good	No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	18	August 20, 1917	$\frac{1}{2}$ acre	.50	Dark-colored agar	Vetch	750 M	90,000 M	Pure	Very good	No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	19	August 21, 1917	$\frac{1}{2}$ acre	.50	White agar	Soybeans	4.5 M	540 M	Not pure. Proteus like bacteria present	Poor	Foul odor. No aeration
Farmogerm—Earl-Thomas Farmogerm Co., Bloomfield, N. J.	20	August 21, 1917	Garden size $\frac{1}{2}$ acre	.50	White agar	Vetches	900,000	108 M	Pure	Good	No bad odor. Aerated container
Farmogerm—McElroy Shepherd Co., Bloomfield, N. J.	21	August 21, 1917	1 acre	1.50	Light-yellow agar	Alfalfa	20 M	600 M	Pure	Good	Aerated container
Alphanos Inoculant—Alphanos Humus Co., New York City.	22	August 25, 1917	1 acre	1.00	Muck	All legumes	.....	.....	Impure	Vetch, alfalfa, peas and clover—Very good. Soybeans—Fair	Finely granulated muck
U. S. Dept. Agriculture—Bureau of Plant Industry, Washington, D. C.	23	August 21, 1917	1 acre	Free	Liquid	Trefoil	30 M	4,500 M	Pure	Trefoil, sweet clover and alfalfa—Good	Uncolored liquid culture. No aeration

Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	24	August 23, 1917	5 acre	5.00	Light agar	Alfalfa	18 M	200 M	Pure	Very good	No odor. No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	25	August 24, 1917	$\frac{1}{4}$ acre	.50	White agar	Alfalfa	12 M	1,440 M	Pure	Good	No odor. No aeration
Alphano Inoculant — Alphano Humus Co., New York City	26	August 28, 1917	1 acre	1.00	Muck	All legumes			Not pure	Soybeans and vetch—Fair. Clover, alfalfa and cowpeas—Good	Finely granulated muck
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	27	August 29, 1917	$\frac{1}{4}$ acre	.50	White agar	Alfalfa	6.3 M	756 M	Pure	Good	No aeration
Nitragin—Nitragin Co., Waterloo, Ia.	28	August 29, 1917	$\frac{1}{4}$ acre		Muck or black soil	Alfalfa	100,000	600 M	Pure (?)	Very good	Guaranteed for 6 months
Nitragin—Nitragin Co., Waterloo, Ia.	29	August 29, 1917	1 acre		Muck or black soil	Vetches, garden peas, field peas	212,000	1,272 M	Fungi present	Vetch—Good. Garden pea—Fair	Guaranteed for 6 months. Aerated container
.....	30	September 8, 1917	Sample	Lost							
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	31	September 8, 1917	$\frac{1}{4}$ acre	.50	Dark-colored agar	Vetch	1.4 M	168 M	Pure	Good	No aeration
Nitrogerm—H. K. Mulford Co., Philadelphia, Pa.	32	September 8, 1917	$\frac{1}{4}$ acre	.50	Light-colored agar	Alfalfa	16 M	192 M	Pure	Good	No aeration. Sweet odor

TABLE 2  
Tests on unofficial samples of commercial legume cultures

NAME OF CULTURE	LABORATORY NO.	PURITY	BACTERIA PER CUBIC CENTIMETER*	EFFICIENCY OF NODULE PRODUCTION	LEGUME	REMARKS
Standard Nitrate Agencias, New York City	1	Pure	5.7 M	Good	Alfalfa	Agar culture price, 50 cents per acre
Mulford Nitrogerm	2	Pure (?)	45 M	Good	Alfalfa	Black agar culture; no aeration
Mulford Nitrogerm	3	Pure (?)	27,000	Very poor	Vetch	Black agar culture average of 1 nodule per plant
Mulford Nitrogerm	4	Sterile	0	Nil	Soybeans	No colonies appeared even after 21 days' inoculation
Earp-Thomas Farmogerm	5	Fungi were present	300 M	Good	Soybeans	Very small colonies developed only after 14 days
Earp-Thomas Farmogerm	6	Pure	90 M	Good	Alfalfa	Aerated container; agar culture
Earp-Thomas Farmogerm	7	Pure	105 M	Good	Alfalfa	.....
Mulford Nitrogerm	8	Fungi present	0	Nil	Soybeans	Culture had been kept 3 months before testing
Mulford Nitrogerm	9	Fungi and foreign bacteria present	3000	Very poor	Soybeans	Only 2 out of 5 plants had nodules (1)
Mulford Nitrogerm	10	Pure	less than 100	Nil	Alfalfa	Culture had been kept in laboratory 3 months when tested

		11	Pure	300 M	Very good	Soybeans	Culture had been stored in laboratory 3 months before test
Earp-Thomas Fermo- germ		11	Pure	300 M	Very good	Soybeans	Culture had been stored in laboratory 3 months before test
Mulford Nitrogerm		12	Fungi present	200 M	Good	Alfalfa	Black agar culture
Alphano Inoculant		13	Fungi and other bacteria present	285,000	Vetch clover and soybeans Good	All legumes	Finely granulated muck
Mulford Nitrogerm		14	Pure	1000	No test	Alfalfa	Stored in laboratory about 2½ months before testing
U. S. Dept. Agr. culture		15	Pure	less than 1000	Very poor: 2 nodules on 5 plants	Vetch	Liquid culture, January 23, 1917
U. S. Dept. Agr. culture		16	Fungi and actinomycetes present	0	Nil	Vetch	Liquid culture. Foul odor. February 10, 1917
U. S. Dept. Agr. culture		17	Pure	1.5 M	Good	Vetch	Liquid culture, May 1, 1917
Earp-Thomas Fermo- germ		18	Pure	300 M	Nil	Soybeans	Sample was stored about 1 month in laboratory
Earp-Thomas Fermo- germ		19	Pure	5000	Poor	Cowpea	Sample stored in laboratory for about 1 month
Earp-Thomas Fermo- germ		20	Pure	25 M	Good	Garden pea	Stored in laboratory for 1 month
U. S. Dept. Agr. culture		17	Pure	175,000	Fair	Vetch	This is sample 17 after storing for 6 months

\* 20 cc. sterile water added to each sample.



## TESTS OF COMMERCIAL CULTURES FOR LEGUME INOCULATION

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Hellriegel and Wilfarth discovered in 1886 that the presence of nodules on the roots of pea plants enabled the plants to make good growth in poor sand. The presence of the nodules was definitely associated with a particular kind of bacteria known as *Bacillus radicicola*. There are many strains of *B. radicicola*, each more or less specific to a particular legume. Pure cultures of the specific strains of *B. radicicola* for all the important leguminous crops have been prepared by the United States Department of Agriculture, state experiment stations and several commercial firms. Experimental tests have been made by the United States Department of Agriculture (2) and New Jersey (1), Georgia (5) and many other experiment stations (3, 4) as to the efficiency of commercial cultures for the inoculation of legumes.

The tests of commercial cultures made have included the enumeration of the bacteria present, the purity of the cultures and the ability of the cultures to inoculate legumes grown in culture media, in field soil and in sterile soil. This report is based on tests where the ability of commercial cultures to inoculate each plant was studied when that proportion of the culture which should go to one plant was added in solution to the seed.

Field tests have shown that three sources of inoculation may exist, namely, the soil, the seed and the introduced culture. In field tests an introduced culture has been termed successful when the plot where the culture was used had a higher per cent of inoculated plants than the check plot. In more exact tests sterile seed, sterile media and sterile soil have been used. These tests have only given evidence of the presence or absence of the specific strain of *B. radicicola* in quantity, for either the seed (or seedling) had been dipped in a solution of the culture or an indefinite quantity of the culture had been added to the seed or medium in which the plant was to be grown.

Table 1 compiled from data furnished by Temple (5) gives the number of viable bacteria of the *B. radicicola* type which he found in commercial cultures calculated in relation to the number of seeds sown per acre.

### THE PRESENT INVESTIGATION

Three objects were sought in the experiments herein reported:

1. To see whether soil or commercial cultures are the more efficient inoculants when each seed planted receives either its exact proportion of the com-

mercial culture or its proportion of the bacteria in the weight of soil used per acre.

2. To see if 1 pound<sup>1</sup> of soil per acre gives as good inoculation as  $\frac{1}{2}$  pound of soil per acre.

3. To see what effect fertilization has on the percentage of inoculation obtained with a specific culture.

#### POT EXPERIMENTS

For objects 1 and 2, pot experiments were conducted using a neutral sterile sandy soil, classified by the Bureau of Soils, of the United States Department of Agriculture, as Wabash sandy loam and locally known as "melon soil."

The soil used contained about 3 per cent of volatile matter. Greenhouse pots 8 inches in diameter, 8 inches deep, and with bottoms 6 inches in diame-

TABLE 1

*B. radiculicola* found in cultures in relation to the number of seed planted

CROP	POUNDS OF SEED PER ACRE	NUMBER SEED PER ACRE	NUMBER BACTERIA PER SEED
Alfalfa.....	20	4,250,000	457*
Crimson clover.....	20	2,925,000	2,882*
Hairy vetch.....	30	450,000	22,593*
Canada field pea.....	60	205,000	14,192*
Cowpea (Whip-poor-will).....	75	180,000	1,250.
Soybean (Mammoth Yellow).....	60	122,000	7,798†

\* Averaged for first five cultures for specific crop reported in table 1 of Ga. Agr. Exp. Sta. Bul. 120.

† Only one culture for cowpeas and four for soybeans given in table 1, Ga. Agr. Exp. Sta. Bul. 120.

ter, were filled with air-dry soil. The pots and soil were sterilized in a Lautenschlager oven with dry heat by bringing the temperature of the soil up to 160°C. in 4 hours' time. The pots were covered with sterile cotton as soon as sterilized and kept sterile until the seeds were planted.

#### *Legumes used and seed sterilization practiced*

Soybeans, sweet clover, cowpeas and hairy vetch were the legumes chosen for the tests. After trying alcohol of various strengths and hydrogen peroxide as sterilizing agents, 3 per cent hydrogen peroxide was chosen as the sterilizing agent. The seeds were placed in sterile 8-ounce dilution bottles

<sup>1</sup> In field inoculation tests from 2½ to 15 pounds of soil per acre have been used. The amounts of the commercial culture used per acre varied from 1 to 9 ounces, so 1 pound and  $\frac{1}{2}$  pound quantities of the soil were chosen for comparison with the commercial cultures.

covered with the hydrogen peroxide and allowed to soak for 1 hour. At the end of the hour's treatment the seeds were washed with five changes of sterile, distilled water, and then allowed to stand in sterile distilled water for 1 hour, after which they were again washed with sterile distilled water. They were taken from the bottles 4 hours later (as planted) with sterile forceps. Seeds of each kind were put in petri dishes with suitable agar and incubated for 7 days at 20°C. No colonies of any kind developed on the agar and the germination was almost perfect.

### *Cultures and soils used for inoculation*

Cultures for the four legumes were obtained from each of four commercial firms. Soil for each legume was obtained from fields where the respective legumes had recently been successfully grown.

### *Diluting the cultures*

The different cultures were diluted so that 1 cc. of the highest dilution made up contained that part of the original culture that would be applied to 1/363,000 of an acre. Three plants were grown per pot. It was decided to grow three plants per pot, since the interspaces between the pots, as they were alternated on the greenhouse bench, would allow plenty of light. Equal numbers of plants were grown in each pot, since this appeared to be the only uniform way of testing all cultures. The average number of seeds planted per acre for crimson clover, hairy vetch, cowpeas and soybeans, as computed from table 1, is 919,250. Our rate of application of commercial culture allowed for 363,000 plants per acre. Table 2 gives data regarding the cultures used and the first dilutions made up.

The amount of material furnished by the various companies for the inoculation of any prescribed area varied considerably. In making the first dilution, material in cans was scraped out into sterile dilution bottles under sterile conditions and put with the amount of water given in table 2. To each bottle culture, containing agar and gelatine-like materials, was added 100 gm. of warm (40°C.) sterile water. The bottle and contents were vigorously shaken for five minutes, and then the contents of the bottle were poured out into a sterile dilution bottle and the culture bottle rinsed out with portions of sterile water. The total water used in each case (including the original 100 gm.) is given in table 2. To insure uniformity of aliquoting, the next dilution was made by putting 25 cc. of the first dilution with that weight of water required to give a dilution such that when multiplied by some multiple of ten the ultimate dilution desired was obtained. All higher dilutions were made by taking 10 cc. of the highest dilution and putting it with 90 cc. of sterile water to make the next higher dilution.



*Planting the seed*

The prepared dilutions were taken immediately to the greenhouse along with the sterilized seed and pots of sterilized soil. The afternoon was cloudy and so special care was not taken to keep the dilutions away from the light.

The cotton covering was removed from a pot of soil, a small hole was made

TABLE 2  
*Cultures and first dilutions made*

TREAT- MENT NO.	PUT UP FOR	WEIGHT OF MATERIAL USED, AND AREA RECOMMENDED FOR USE		AMOUNT OF H <sub>2</sub> O WITH WHICH MATERIAL WAS PUT FOR FIRST DILUTION
		gm.	acres	gm.
1*	Soybeans .....	131.0	$\frac{1}{2}$	183.4
2	Sweet clover .....	130.0	$\frac{1}{2}$	182.0
3	Cowpeas .....	125.1	$\frac{1}{2}$	175.2
4	Hairy vetch .....	132.5	$\frac{1}{2}$	185.5
5	Soybeans .....	15.0	$\frac{1}{2}$	195.0
6	Sweet clover .....	15.1	$\frac{1}{2}$	196.3
7	Cowpeas .....	20.0	$\frac{1}{2}$	260.0
8	Hairy vetch .....	15.3	$\frac{1}{2}$	198.9
9	Soybeans .....	26.1	$\frac{1}{2}$	156.6
10	Sweet clover .....	22.4	$\frac{1}{2}$	134.4
11	Cowpeas .....	15.8	$\frac{1}{2}$	205.4
12	Hairy vetch .....	27.7	$\frac{1}{2}$	166.2
13	Soybeans .....	35.5	1	163.3
14	Sweet clover .....	29.5	1	135.7
15	Cowpeas .....	33.5	1	154.1
16	Hairy vetch .....	31.3	1	144.0
17	Soybean soil .....	50.0	$\frac{1}{8}$	200.0
18	Sweet clover soil .....	50.0	$\frac{1}{8}$	200.0
19	Cowpea soil .....	50.0	$\frac{1}{8}$	200.0
20	Hairy vetch soil .....	50.0	$\frac{1}{8}$	200.0
21	Soybean soil halved .....	†		
22	Sweet clover soil halved .....			
23	Cowpea soil halved .....			
24	Hairy vetch soil halved .....			

\* First four cultures from same company, second four cultures from another company and so on to and including treatment no. 16.

† Made by diluting part of final dilution of nos. 17, 18, 19 and 20 with an equal volume of water.

in each third of the surface of the soil by means of a sterile stick, three seeds were put into each of these holes, then 1 cc. of the culture solution was put on each lot of three seeds, and finally the seeds were covered with soil with the use of the sterile stick. Three pots were planted in this way for each of the twenty-four treatments and also three check pots for each of the legumes. In planting the checks, 1 cc. of sterile water was added in place of 1 cc. of the

dilution of a culture. Enough sterile distilled water to wet the surface was slowly added to each pot. The pots were placed in saucers which were filled with sterile distilled water. The soil was kept moist both by surface and subsoil watering with sterile distilled water until the plants were harvested seven weeks later. When the plants had come up they were thinned to three per pot (one in each third). Care was taken to have the most uniform plants for the three triplicate pots remaining. The seedlings taken out were removed by pinching off at the surface of the soil. Pieces of heavy galvanized wire were inserted as supports for the plants whenever they showed a tendency to fall down. The data secured at the time of harvesting are given in table 3.

Table 3 shows the following:

1. The seeds and soil were sterile, for only in one case out of a possible thirty-six chances did the checks show inoculation.
2. In only five out of the twenty-seven treatments did all nine of the plants survive to harvest time. (In all cases except two cowpea pots the plants were thinned, as previously noted, to three plants per pot.)
3. The inoculated plants had a higher average height than those uninoculated.
4. Both the commercial cultures and the soil in quantities as used were insufficient inoculants for soybeans, cowpeas and hairy vetch.
5. All cultures gave 100 per cent inoculation of the sweet clover.
6. The percentage of stand at the time of harvest was 95.1 for sweet clover, 79.4 for the hairy vetch, 52.4 for the soybeans and 52.4 for the cowpeas.

The following notes were recorded concerning the inoculation:

The one soybean plant inoculated had one nodule about the size of a radish seed.

The sweet clover plants receiving inoculating material all contained large numbers of small nodules scattered throughout the root systems. The inoculated check plant had one little clump of nodules, the size of a pin head, 3 inches below the crown.

One inoculated vetch plant, which was 20.5 inches high, carried many large clumps of nodules. One plant 8.0 inches high had one small nodule the size of a radish seed, and the third plant 14.0 inches high, had one clump of nodules.

#### GREENHOUSE PLOT TESTS

For object 3 of this investigation, soybeans were grown in greenhouse plots 3 by 5 feet with different fertilizer treatments on two soils, a bank sand and a brown silt loam. The plots had been twice cropped to lettuce, being fertilized 7 months and again 4 months previous, as given in table 4. The seed used was commercial, of the variety Early Brown. To supplement any *B. radiculicola* present in the soils and on the seeds, a commercial culture of the same brand as that used in treatment no. 1 in table 2 was secured. The

culture was put with enough sterile water to moisten 15 pounds<sup>2</sup> of seed and the water and culture mixture poured over the 15 pounds of seed (contained in a large dish-pan). The mass was mixed over and over until no seed could be found which did not have black spots of the soil in the commercial cul-

TABLE 3

*Results of commercial cultures and soil for legume inoculation with sterile seed and sterile soil*

CROP	TREATMENT NO.	NUMBER OF PLANTS LEFT AT HARVEST	AVERAGE HEIGHT OF PLANTS (EXTENDED)	NUMBER OF PLANTS INOCULATED	AVERAGE HEIGHT OF INOCULATED PLANTS (EXTENDED)
			<i>inches</i>		<i>inches</i>
Soybeans.....	1	6	10.0	0	10.0
	5	2	9.9	0	
	9	4	8.9	0	
	13	5	9.7	1	
	17 (1 lb. soil)	6	9.3	0	
	21 ( $\frac{1}{2}$ lb. soil)	6	10.0	0	
	25 (check)	4	9.3	0	
Sweet clover.....	2	8	7.9	8	7.9
	6	9	5.2	9	5.2
	10	9	5.9	9	5.9
	14	8	6.4	8	6.4
	18 (1 lb. soil)	9	8.1	9	8.1
	22 ( $\frac{1}{2}$ lb. soil)	8	6.8	8	6.8
	26 (check)	9	3.4	1	7.0
Cowpeas.....	3	5	3.1	0	
	7	5	3.7	0	
	11	6	3.5	0	
	15	5	4.4	0	
	19 (1 lb. soil)	4	3.0	0	
	23 ( $\frac{1}{2}$ lb. soil)	3	3.8	0	
	27 (check)	6	3.2	0	
Hairy vetch.....	4	6	8.1	0	20.5
	8	7	7.5	1	
	12	8	6.4	0	
	16	6	6.0	1	8.0
	20 (1 lb. soil)	8	5.8	0	14.0
	24 ( $\frac{1}{2}$ lb. soil)	9	7.1	1	
	28 (check)	6	5.8	0	

ture clinging to it. The seeds were planted 2 inches deep and 4 inches apart in drills 8 inches apart. Uninoculated but non-sterile seeds from the same bag as those inoculated were planted in the check plots. The plants were

\* More culture to seed than normal to insure inoculation.

harvested when the majority of the pods were fairly well filled. The inoculation results are given in table 4.

The table shows that both soil type and fertilization had an effect on inoculation. In most cases the calculated effects of the additions of nitrogen in the form of sodium nitrate decreased the per cent of inoculated plants.

TABLE 4  
*Fertilizer treatments and percentages of plants inoculated under different conditions*

FERTILIZER TREATMENT PER ACRE*	PERCENTAGE INOCULATION		
	Sand, seed unin- oculated†	Brown silt loam	
		Seed unin- oculated ‡	Seed inocu- lated §
	per cent	per cent	per cent
400 lbs. A. P.....	24	3	85
800 lbs. A. P.....	39		
133 lbs. NaNO <sub>3</sub> , 400 lbs. A. P.....	29	27	89
266 lbs. NaNO <sub>3</sub> , 800 lbs. A. P.....	15		
400 lbs. NaNO <sub>3</sub> , 133 lbs. A. P.....	47	11	75
800 lbs. NaNO <sub>3</sub> , 266 lbs. A. P.....	5		
133 lbs. NaNO <sub>3</sub> , 400 lbs. A. P., 200 lbs. KCl.....	84	36	81
266 lbs. NaNO <sub>3</sub> , 800 lbs. A. P., 400 lbs. KCl.....	13		
400 lbs NaNO <sub>3</sub> , 200 lbs. KCl.....	0	15	69
800 lbs. NaNO <sub>3</sub> , 400 lbs. KCl.....	35		
400 lbs. NaNO <sub>3</sub> .....	6	15	70
800 lbs. NaNO <sub>3</sub> .....	5		
20 T. M., 133 lbs. NaNO <sub>3</sub> , 400 lbs. A. P.....	3	8	74
20 T. M.....	4	22	70
Check.....	5	46	63
Average.....		20	75

\* NaNO<sub>3</sub> = sodium nitrate; A. P. = acid phosphate; KCl = potassium chloride; 20 T. M. = 20 tons manure.

† Average number of plants per plot 41.

‡ Average number of plants per plot 38.

§ Average number of plants per plot 50.

#### DISCUSSION

The number of organisms calculated for each seed in table 1 was so great that we were led to believe that the successes and failures of commercial cultures could not be regularly attributed to lack of organisms producing the *B. radiculicola* type of colonies on petri plates. It is impossible to tell one strain of *B. radiculicola* from another by plate culture characteristics, and hence the only real method of testing the culture is to see if it will produce nodules on the legume plant when distributed evenly over the area recommended.

There is great variation in the quantities of commercial cultures sold for inoculating the same area. The history of the culture and its exact purity are rarely known. Cultures for a specific legume may be more uniformly active than those for other legumes. All our sweet clover cultures proved out. The legume seeds evidently carry the specific strains of *B. radicicola* necessary, for in our tests in the greenhouse plots considerable inoculation was secured without the commercial culture. The statements of various workers that the check plants usually contain many nodules bears this out. The cultures and soil are never as uniformly distributed over an acre of ground as was done in our tests, therefore many of the uninoculated spots in fields evidently are due to the fact that plants in some other spots having inoculation received more than their share of the inoculant. When the inoculating material was diluted and applied so that each seed received its proportion the following resulted:

The sweet clover cultures from the four firms and the soil in the two amounts gave 95.1 per cent of a stand and perfect inoculation, while the soil and cultures were unsatisfactory for the other three legumes. *B. radicicola* has been found to persist for many years in soil and for many months on the seed. Commercial cultures and soil for seed inoculation should be used in amounts that give enough bacteria to furnish all the inoculation rather than in quantities to supplement the organisms present on the seed and in the soil.

#### SUMMARY

1. With sweet clover both soil and commercial cultures, when diluted so that each seed obtained either its exact proportion of the commercial culture or its proportion of the bacteria in the weight of soil used per acre, gave successful inoculation. Soil and cultures were equally efficient, as they gave 100 per cent inoculation.

2. With soybeans, cowpeas and hairy vetch, satisfactory inoculation was not secured with either commercial cultures or soil.

3. Both quantities of soil failed to produce inoculation with three of the legumes. Therefore, the double quantity of soil can not be credited as better than the single.

4. A commercial culture, when applied at double rate to soybean seeds which were sown in greenhouse plots, gave an average percentage inoculation of 75 compared with 20 per cent for similar plots receiving no inoculating material.

5. Fertilization with nitrate of soda tended to reduce the percentage of inoculation secured.

6. Larger quantities of commercial cultures and soil than those used in these tests would be necessary to furnish satisfactory inoculation over the entire area for which the culture was put up.

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# PLATE 1

FIG. 1. Vetch plants showing effect of inoculation: plants at left inoculated; check plants at right.

FIG. 2. Plants at left inoculated by commercial cultures; plants at right inoculated by soil; center plants uninoculated checks.



FIG. 1



FIG. 2





# THE EFFECT OF INOCULATION, FERTILIZER TREATMENT AND CERTAIN MINERALS ON THE YIELD, COMPOSITION AND NODULE FORMATION OF SOYBEANS<sup>1</sup>

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## THE EFFECT OF INOCULATION ON THE YIELD AND COMPOSITION OF SOYBEANS

It has been shown many times that inoculation increases both the yield and the protein content of soybeans, but little or no data have been presented to show the effect upon the oil content.

Lipman and Blair (28) showed that in well inoculated soybean plants the per cent of nitrogen was greater than in uninoculated or in poorly inoculated plants; and that the yield also was higher. The West Virginia (5), Michigan (47), Kansas (6), and Wisconsin (9) experiment stations also have shown that a good inoculation increases the yield as well as the protein content of the plant. Some investigators have reported slight, or no, increase in yield due to inoculation. Among these are the Nebraska (23) and Wisconsin stations. The Illinois (51) station showed that inoculated cowpeas were much richer in nitrogen than uninoculated cowpeas. Not only is it a well established fact that inoculation increases the yield and protein content of legumes in general, but as Lipman (27) and Lyon and Bizzell (34) have shown, the association of non-legumes with legumes in mixed cultures results in an increased percentage of protein for the former. That this fact was well known and put into actual practice for centuries past, was shown by Dr. Lipman.

Although much work has been done on the effect of inoculation on the protein content of legumes and of soybeans in particular, but little has been done on the factors causing variations in the oil content of the seeds.

Perhaps the most work done on the effect of inoculation upon the composition of the soybean plant was done by Lipman and his associates (28, 29), Fred (9), and Smith and Robinson (45). These workers show that a substantial increase in protein results from inoculating soybeans. The former two writers also showed that liming increased the percentage of protein in soybeans.

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Mention has been made a few times that the oil content of soybeans decreases as the protein content increases. Among the authors who have mentioned this phenomenon are Robert (38) and Allison (3). They claim that high oil and high protein content do not go together in soybeans.

Grantham (14) in 1912 states:

Very high oil and very high protein content in soybeans do not seem to be closely correlated. The variety yielding the most protein was lowest in oil. Whether the high per cent of protein and oil can be increased by selection and breeding remains to be seen, as little or no work has been done along this line.

The considerable variation in composition among varieties offers an opportunity for developing strains of soybeans for specific purposes.

Robert (39) concludes from the results of his analyses of soybeans that high oil and protein content are not correlated, but that high protein yielding varieties have a low oil content. Most of the investigators just quoted are cognizant of this fact, but little or no work has been done on the effect of inoculation upon the oil content of soybeans.

The author studied this problem in both greenhouse pot experiments and in field plots. The variety of soybean used was the "Black Eyebrow," a very promising variety for New Jersey. Morse (36), of the United States Bureau of Plant Industry, characterizes it as follows:

The seeds are black and yellow. It is an early variety obtained from Manchuria and maturing slightly earlier than Ito San, excelling the latter in both hay and seed production. It is most suitable as a grain variety in the northern states.

Jenkins (20) of Connecticut states: "Black Eyebrow soybeans yielded 21.6 bushels per acre of seed and matured in 109 days. Only one variety matured earlier, and that one by only two days." Lipman and Blair (29), of the New Jersey station, state that it requires about 120 days for maturity. The 1915 crops yielded only 10.8 bushels of seed per acre with a protein content of 40.5 per cent.

#### *Methods used for the analysis of soybeans*

The samples were partly dried to facilitate grinding and then ground in a small power mill to a flour. All samples were ground to pass a 40-mesh sieve as it was found that the oil was incompletely extracted from several samples which were more coarsely ground. Good duplicate determinations were obtained by using material for analysis which had passed a 40-mesh sieve. The Soxhlet method of extraction was used for the determination of the oil. Electric light bulbs were used to furnish the necessary heat. Extractions were run 8 hours, as it was found that practically all of the oil could be extracted in that length of time. The flasks containing the oil were dried to constant weight in an electric oven at a temperature of 100°C. It

was noticed that if the flasks remained in the oven too long an increase in weight took place. This was evidently due to the absorption of oxygen by the oil, as it became thicker and tougher the longer it remained in the oven. From 30 to 50 minutes' drying was found to give satisfactory results.

The nitrogen content of soybeans was determined by the Gunning modification of the Kjeldahl method; namely, by using for a half-gram sample of bean flour, 25 cc. of concentrated sulfuric acid and 5 gm. of potassium sulfate. A modification was further made by adding to each flask about 1 to 2 gm. of copper sulfate in addition to the above chemicals. It was found to facilitate digestion greatly. The function of the copper sulfate is probably that of a catalyzer, although it may assist in the oxidation of the organic matter by taking part in the reaction. A complete digestion may be carried out in this way in from 20 to 30 minutes.

### *Pot experiments*

The pots used were 10-pound glazed earthenware pots. The soil was a virgin Sassafras sandy loam containing no nitrogen-fixing organisms of the soybean variety. The fertilizer treatment was 2 gm. of acid phosphate, 2 gm. of muriate of potash, and 5 gm. of ground limestone per pot. The seeds, after being sterilized with  $\text{HgCl}_2$  (1:1000) and thoroughly washed for some hours in distilled water, were inoculated with soil in varying quantities and commercial cultures, and planted at the rate of 8 to the pot. Pots 19 to 22 were inoculated with a pure culture of *Bacillus radicicola*, isolated a few days before from a soybean nodule, and grown since that time on Ashby's nitrogen-free medium.

These data show that there is a considerable variation, with the different methods of inoculation, in the yield of total dry matter, the number of nodules per plant, and the per cent of oil and protein in the seeds of the plants. The four check pots agreed very well in yield of total dry matter, at about 9 gm. per pot. Pots 11 and 12 inoculated with Mulford's Nitrogerm also agreed with the checks, showing that little or no inoculation had been produced. Pots 3 to 10 form an interesting series. It appears from the data obtained that the yield of total dry matter, number of nodules and per cent of protein vary directly as the amount of soil added up to 5 gm., when the maximum is reached. Ten grams of inoculated soil per pot did not give increases over 5 gm. The other cultures rank about the same as regards nodule production, yield and per cent of protein. Apparently, the most efficient culture of all was the freshly isolated culture of *B. radicicola* from soybean nodules. In the four pots where used it gave increased yield and nodule production, and high protein and low oil content. The inoculating efficiency of two soils both containing the soybean organism, appears to vary, since almost twice as many nodules were produced in one case as in the other. The per cent of oil and protein is proportionally large or small ac-

cording as the inoculation was good or poor. The oil varies from 17.7 to 22.5 per cent, the greatest amount being present in the checks, and the lowest in those pots having the most thorough inoculation, and consequently the greatest number of nodules per plant.

TABLE 1  
*Effect of inoculation upon the yield and composition of soybeans*

POT	NATURE OF INOCULATION	TOTAL DRY MATTER	AVERAGE TOTAL DRY MATTER	AVERAGE NUMBER NODULES PER PLANT	PER CENT PROTEIN IN SEED	PER CENT OIL IN SEED
		gm.	gm.			
1	Check: no inoculation	8.5	8.90	0	38.6	21.9
2		9.3				
3	1 gm. soil from soybean field	12.7	13.15	2.3	40.0	20.7
4		13.6				
5	2 gm. soil from soybean field	14.9	14.80	5.2	41.2*	19.1*
6		14.0				
7	5 gm. soil from soybean field	17.7	17.25	24.0	41.9	18.0
8		16.8				
9	10 gm. soil from soybean field	17.6	17.10	31.0	41.9	17.7
10		16.6				
11	Mulford's Nitrogerm	9.2	8.15	0.4	39.0	22.4
12		7.1				
13	Earp-Thomas Farmogerm	18.1	18.10	25.0	41.9	19.0
14		18.1				
15	Standard Nitrate Agencies' culture	17.3	17.75	18.0	42.0	18.1
16		18.2				
17	U. S. Dept. Agr. Culture	18.6	18.25	21.0	41.6	18.4
18		17.9				
19	Freshly-isolated pure culture: 1 cc.	20.1	19.90	24.1	40.2	17.9
20		19.7				
21	Freshly-isolated pure culture: 10 cc.	20.1	20.50	26.0	41.7	17.9
22		20.9				
23	2 gm. soil from soybean field no. 2	17.2	16.80	9.1	41.6	19.6
24		16.4				
25	Check: no inoculation	9.0	9.15	0.8	38.2	22.5
26		9.3				

\*Pot 5 only; pot 6 was lost.

From this experiment it could be concluded that in a rather poor sandy soil inoculation decreased the oil content of soybeans in proportion to the thoroughness of the infection. In the same way the protein content is increased.

*Field experiments to show effect of inoculation upon yield and composition of soybeans*

Since such interesting data were obtained from pot experiments it was decided to continue the experiment in the field. The soil was an acid Sassafras sandy loam, which had never grown soybeans and which was shown to contain no *B. radicicola* (variety soybean) by culture of soybeans on the land. The plots were 5 by 10 feet, or 0.001141 acre in size. The vegetation existing on the land when broken was sparse, consisting principally of wild celery, crab-grass and Canada blue-grass. The variety of soybeans used was the Black Eyebrow. Plots were planted in duplicate in all cases with an intervening space of 12 inches. Exactly the same fertilizer treatment was given each duplicate plot. This consisted of applications at the acre rate of 400 pounds of acid phosphate, 200 pounds of muriate of potash and 2000 pounds of ground oyster shells in excess of the lime requirement as determined by the Veitch method. The soil used for inoculating some of the plots had grown a luxuriant crop of soybeans the year before, the plants being well supplied with nodules. This soil was spread over the plots evenly and worked in with a rake. The commercial culture used was Mulford's Nitrogerm. This was diluted according to directions and the beans allowed to soak 2 hours in a dark room. They were planted while still moist and covered immediately. The first nodules were noticed after 12 days on plot 72A. From the beginning of the experiment the inoculated plots were evident from their dark color and increased leafiness. The seeds had fully matured in 95 days, and after drying, weighing, threshing and cleaning, the beans were analyzed for oil and protein content. The roots, tops and straw also were weighed and analyzed, but the results are given in another paper. Counts of nodules on the roots also were made.

The data in table 2 show that the yield of soybeans is very materially increased by inoculation, and that this increase becomes larger with the thoroughness of the infection. The duplicates in some instances do not agree very well, but the large number of check plots without any inoculation show that any increases in crop yields are undoubtedly due to the presence of the nitrogen-gathering bacteria. Not only total dry matter was increased, but also the seed and straw yields showed substantial increases over the checks.

As in the pot experiments, Mulford's Nitrogerm did not inoculate the soybeans. The number of nodules per plant and yield of total dry matter and seed increased with the amount of soil used for inoculation. The field experiment substantiated the indications procured in the pot experiments.

*Effect of inoculation upon the composition of soybeans*

The seed from these plots was carefully sampled, dried, ground and analyzed for oil and protein content. The data obtained are given in table 3. Only plot numbers are given, as reference can easily be made to table 2 which shows the treatments of the several plots.

TABLE 2  
*Effect of inoculation upon the yield of soybeans*

PLOT NO.	INOCULATING MATERIAL	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	TOTAL DRY MATTER INCREASE OVER CHECKS	SEED INCREASE OVER CHECKS	NUMBER NODULES PER PLANT*
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	
70	Check: no inoculation	3.11	1.16	1.95	2.85	1.06			0.1
70A		2.59	0.95	1.64					
71	Soil: 11 lb.	3.83	1.68	2.15	3.33	1.50	0.54	0.48	5.0
71A		2.83	1.31	1.52					
72	Soil: 5 lbs.	3.82	1.78	2.04	3.53	1.75	0.79	0.08	10.2
72A		3.24	1.71	1.53					
73	10 cc. Mulford Nitro-germ	2.30	0.85	1.52	2.24	0.86	-0.45	-0.05	0
73A		2.11	0.90	1.21					
74	Check: no inoculation	3.06	1.02	2.04	2.62	0.89			0
74A		2.17	0.76	1.41					
75	30 cc. Mulford Nitro-germ	2.72	1.07	1.65	2.65	0.98	-0.26	-0.04	0.3
75A		2.57	0.89	1.68					
76	10 cc. Nitrogerm - 1 lb. soil	3.92	1.90	2.02	3.98	1.84	1.26	0.83	7.2
76A		4.05	1.78	2.27					
77	Check: no inoculation	2.95	1.13	1.82	3.00	1.10			0.3
77A		3.05	1.20	1.85					
78	Soil: 3 lbs.	3.67	1.76	1.91	3.75	1.64	1.25	0.48	7.0
78A		3.83	1.51	2.32					

\* Nodule counts were taken when plants were 32 days old.

Table 3 shows that the inoculation of soybeans increases the per cent of protein and decreases the per cent of oil in the seeds. The average increase in protein content for the eight inoculated plots is 7.04 per cent and the average decrease in oil content due to inoculation in these same eight plots is 3.18 per cent.

In connection with the oil determinations, crude drying tests were made from all of the samples to see if the quality of the oil varied, but as far as

was shown by the simple drying tests made, there is little if any difference in the oil from these beans. The method of procedure was to warm the flasks containing the oil residue to decrease its viscosity, then to make smears of this oil on pine boards by means of small brushes. These smears were examined several times daily. Because of the lack of uniformity in procedure, and in the treatment of the oil samples (some had to be dried longer than others to become constant in weight), too much emphasis cannot be placed on these

TABLE 3  
*Effect of inoculation upon the composition of soybeans*

PLOT	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECKS	INCREASE IN PROTEIN OVER CHECKS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
70	19.1	36.0	19.20	36.10		
70A	19.3	36.2				
71	16.8	39.4	16.90	39.95	-3.77	5.65
71A	17.0	40.5				
72	18.1	44.6	18.55	43.80	-3.22	9.50
72A	19.0	43.0				
73	20.9	36.8	21.10	34.70	+0.33	0.40
73A	21.3	32.6				
74	21.2	33.3	21.95	33.30		
74A	22.7	33.3				
75	22.0	33.3	21.80	33.45	+1.03	0.05
75A	21.6	33.6				
76	17.3	38.5	17.65	38.75	-3.12	4.45
76A	18.0	39.0				
77	20.7	33.3	21.15	33.85		
77A	21.6	34.4				
78	18.3	42.7	18.05	42.85	-2.62	8.55
78A	17.8	43.0				

results. Some samples were darker colored than others, but as a rule the differences were slight.

*Effect of liming the soil upon the yield and composition of soybeans*

Extensive researches have been carried on in this field of work. The most complete are those of Lipman and Blair (29, 30, 32) who have made a number of tests, and in every case found the yield and protein content increased



as a result of liming. They made use of both vegetative greenhouse experiments and field plot tests in their work. On an average for three years, the increase in protein content due to liming was 3.06 per cent. The increase in yield varied with the several varieties from 0 to 11 bushels; with an average of about 5 bushels per acre for a 3-year period. Liming increased nodule production 42 per cent. It was estimated that a crop of soybeans will fix about 65 pounds of nitrogen per acre per year. In another article, Lipman and his coworkers (33) showed that an application of 1000 pounds of lime per acre was as beneficial as 4000 pounds for soybeans.

Fred and Graul (9) working with an acid Colby silt loam, as a result of their investigation state:

Inoculation is very beneficial to soybeans, but liming alone did not cause any very consistent gain in total nitrogen; although where both lime and inoculation were used there was a slight gain in yield and total nitrogen, but hardly enough to warrant recommending lime for soybeans.

They found small applications of calcium carbonate to be as beneficial as larger applications for the common legumes when grown on acid soils. Large applications were injurious.

Duggar and Funchess (71) found that liming increased the yield of soybeans 49 per cent. Hopkins (16), Frear (8) and Lipman (33) have reported results showing small quantities of lime to be as beneficial as larger amounts in the case of various legumes.

Prianishnikov (38) found small dressings of calcium carbonate beneficial to lupines, but large applications were injurious. Ulbricht (48) and Khandurin (22) also showed the same point with other legumes. Kassovich and Althausen (24) working on the acid podzol soils of Russia, found that plants highly sensitive to acidity, as mustard and clover, were helped the most by additions of lime sufficient to neutralize the soil acidity, but were most injured by excessive amounts of lime.

Schulze (41) found 1 per cent of calcium carbonate in soils very injurious to lupines and serradella, and 5 per cent was sufficient to prevent growth. Even 0.5 per cent was decidedly injurious.

Kopeloff (25) has recently shown that the effectiveness of ground limestone is partly measured by its fineness of division, hence one ton of 200-mesh material may act in the same manner as three or four tons of coarsely ground limestone. Abbott (1) reports an increase in yield of soybeans due to the use of lime of 7 bushels per acre.

This literature concerning the use of lime for soybeans could easily be augmented, but enough has been cited to show that liming increases the yield of most legumes, that small amounts are nearly as good as large amounts, and that, in general, liming increases the yield and protein content of the seed.

*Pot experiments to show the influence of lime upon the yield and composition of soybean seeds*

Glazed, round, earthenware pots holding 10 pounds of soil were used in this experiment. The soil was acid Sassafras sandy loam. The lime requirement according to the Veitch method was 6000 pounds of  $\text{CaO}$  per acre. The ground limestone used was 40-mesh material, although a portion (about 10 per cent) did not pass a 40-mesh sieve. Seven seeds of the Black Eyebrow variety were allowed to mature in each pot. The moisture was kept at optimum as nearly as possible. Two grams each of acid phosphate and muriate of potash were added to each pot, thus making the limestone the only variable factor. Inoculation of the seed was made from a suspension of crushed soybean nodules. Table 4 gives the data obtained in this experiment.

Table 4 shows that both ground limestone and burnt lime increase the yield of total dry matter of soybeans on an acid soil. In some cases the crop yield is nearly doubled, and in every case, except where there was a tremendous excess of  $\text{CaCO}_3$  in the soil, there was a material increase. Ground limestone and burnt lime seem to be about equally efficient in increasing crop growth and in nodule production. It is seen at a glance that small quantities of lime or ground limestone are practically as efficient as larger amounts in stimulating plant growth and nodule production, and in increasing the protein content. The oil content of the seeds varies considerably. This is to be expected since in some cases only a few grams of seeds were obtained from a pot and these might well vary in composition. The reason analyses were not determined on the separate pots was that the yield of seed was not great enough from a single pot to insure an accurate analysis, hence the seeds from duplicate pots were mixed and then ground and analyzed. The counts of nodules are not absolutely accurate, as by the time the plants had matured some of the nodules had begun to decay, making the counting difficult at times. As in the case of former experiments, the oil content of soybean seeds decreases as the protein increases, and vice versa. The highest percentage of oil was obtained from the check pots to which no lime was applied. Ordinarily, the oil content would probably not be much affected by liming in addition to inoculation, but on the very acid soil which was used in this experiment the nitrogen-gathering bacteria were not very efficient, as is shown by the lower number of nodules on the roots of the check plots. From this experiment it may be concluded that liming increased the total dry matter, the number of nodules per plant, and the protein content of the seed of soybeans grown in an acid soil. Little or no differences are evident whether burnt lime or ground limestone is used to correct the soil acidity. Large quantities of either of these materials had an inimical effect on plant growth, Soybeans do not seem to require much lime in the soil for their best growth, as when only sufficient burnt lime or ground limestone was added to the soil

TABLE 4

*Effect of lime and ground limestone upon yield and composition of soybeans*

POT NO.	TREATMENT: 2 GM. A. P. AND KCl	TOTAL DRY MATTER	AVERAGE TOTAL DRY MATTER	NUMBER OF NODULES	PROTEIN IN SEEDS	OIL IN SEEDS
		gm.	gm.		per cent	per cent
1	Checks: no limestone	10.7	10.90	18.3	36.0	22.1
2		11.1				
3	Acidity half neutralized	17.3	17.15	31.1	39.7	18.5
4		17.0				
5	Acidity neutralized	17.6	17.85	27.2	42.6	18.2
6		18.1				
7	1000 pounds excess limestone	19.4	18.85	26.1	44.0	18.5
8		18.3				
9	2000 pounds excess limestone	19.2	18.40	31.7	43.6	19.6
10		17.6				
11	5000 pounds excess limestone	14.7	15.85	24.3	45.3	18.2
12		17.0				
13	10,000 pounds excess limestone	13.3	12.15	25.9	45.8	17.3
14		11.0				
15	Check: no limestone	11.7	11.00	13.7	37.2	21.6
16		10.3				
17	Acidity half neutralized with CaO	18.7	19.05	33.6	41.7	19.0
18		19.4				
19	Acidity neutralized with CaO	18.0	17.55	31.4	43.0	18.7
20		17.1				
21	1000 pounds excess CaO	18.3	18.75	28.3	43.4	18.0
22		19.2				
23	2000 pounds excess CaO	15.7	14.80	28.3	43.9	18.6
24		13.9				
25	5000 pounds excess CaO	13.3	13.30	21.2	44.6	17.6
26		13.3				
27	Check: No CaO	10.6	10.85	15.4	36.9	21.7
28		11.1				

to neutralize one-half of the acidity present (Veitch method), maximum crop yields were obtained.

Liming increased the per cent of protein and decreased the per cent of oil in soybeans grown on an acid soil. This is not a weighty argument against liming, because the greater amount of oil would be produced in a full crop of soybeans rather than in half a crop or two-thirds of a crop, which would be obtained on unlimed soil, in spite of the latter's greater oil content. In other words, the greater oil percentage in unlimed soybeans or in uninoculated plants does not overbalance the increased yield due to liming and inoculation.

As in the case with the inoculation experiments, the vegetation results were checked up in field plots the following summer. Plots 5 by 10 feet, as previously described in this paper, were used. The soil was an acid Sassafras sandy loam, of rather poor crop-producing power. Only enough plots were available to test the effect of ground limestone in the field, hence the burnt lime was not used. The similarity of results of the vegetation experiments, however, led the author to believe that the results would have closely simulated those obtained for ground limestone. All of the plots were well inoculated except plot 79, which was neither limed nor inoculated, and plot 80. The form of lime used in this experiment was ground oyster shells, the greater part of which passed a 10-mesh sieve. All of the plots received a fertilizer dressing equivalent to 400 pounds of acid phosphate and 200 pounds of muriate of potash per acre. The inoculation was performed by adding 3 pounds of well infected soil to each plot, and working in with a rake. The beans were planted June 17, 1916, the variety used being the Black Eyebrow. The beans were kept well cultivated during the summer, and were harvested September 21, 1916. Thus 96 days were required for maturity. The limed plots matured about 6 to 8 days later than the unlimed check plots. The plants of the former were also much larger and leafier.

The data are given in tables 5 and 6.

Upon examining tables 5 and 6 it is seen that the field experiments closely check up the vegetation experiments with limestone, already discussed. As in the latter, the field experiments show that liming the soil is of great benefit in increasing the yield of total dry matter (tops) and seeds. The application of enough lime to neutralize one-half of the soil acidity gave an increased crop yield of 30 per cent; where the acidity was exactly neutralized 37 per cent; and where there were 6000 pounds of ground oyster shells in excess of the lime requirement, an increase of 42 per cent was noted. From these figures it is at once seen that it will not pay to add large quantities of lime to soils even though they are very acid, if soybeans are to be grown. Although the crop yields continue to increase when lime is applied in excess of the amount required to neutralize the soil acidity, yet these increases are small, and when we consider the cost of lime, application, and the amount leached away in the drainage water each year, it is not a paying investment to add large quan-

tities to a field. It is far better to make small applications of, say, 1000 to 3000 pounds of ground limestone every two or three years.

An interesting point is brought out in the results obtained from plots 79, 80 and 81. They also show the relative importance of inoculation and liming

TABLE 5

*Effect of varying quantities of ground oyster shells upon the yield and composition of soybeans*

PLOT NO.	TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
79	Check (I): No inoculation, no lime	1.92	0.80	1.10	1.96	0.76	-0.44*	-0.33	0.†
79A		2.03	0.73	1.30					
80	Check (II): Lime, no inoculation	2.95	1.13	1.82	3.00	1.16	+0.60	0.06	0.3
80A		3.05	1.20	1.85					
81	Check (III): No lime, inoculation	2.30	1.14	1.16	2.30	1.05			1.5
81A		2.30	.96	1.34					
82	Acidity half neutralized	3.98	1.97	2.01	3.58	1.82	1.18	0.73	7.4
82A		3.17	1.67	1.50					
83	Acidity just neutralized	4.04	1.88	2.16	3.87	1.76	1.42	0.64	10.0
83A		3.70	1.63	2.07					
84	2000 lbs. excess CaCO <sub>3</sub>	3.67	1.76	1.91	3.75	1.64	1.25	0.48	7.0
84A		3.83	1.51	1.32					
85	4000 lbs. excess CaCO <sub>3</sub>	3.99	1.88	2.11	3.64	1.76	1.09	0.65	7.5
85A		3.29	1.64	1.65					
86	Check: No CaCO <sub>3</sub>	2.65	1.26	1.39	2.65	1.23			1.4
86A		2.65	1.19	1.46					
87	6000 lbs. excess CaCO <sub>3</sub>	4.46	2.06	2.40	4.25	1.86	1.57	0.62	12.4
87A		4.03	1.66	2.37					
88	10,000 lbs. excess CaCO <sub>3</sub>	4.52	2.20	2.30	4.28	1.93	1.38	0.81	15.3
88A		3.66	1.92	1.74					
89	Check: No CaCO <sub>3</sub>	2.87	1.35	1.52	2.75	1.28			1.9
89A		2.63	1.21	1.42					

\* Check plot III (81-81A, 86-86A, and 89-89A), are used as the basis of calculation.

† Nodule counts were taken when the plants were 33 days old.

for soybeans. From the data it appears that inoculation is the less important, as plot 80, which has lime but no inoculation, gave an increase in crop yield of 30 per cent over plot 81 which had no lime but which was in-

oculated. However, too much emphasis should not be placed on this single experiment, as soil conditions and different varieties of soybeans might give other results. That liming alone, or inoculation alone, gives an increase over the no-lime, no-inoculation check, is brought out in the tables. It is

TABLE 6  
*Effect of limestone upon the composition of soybean seed*

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE OF OIL OVER CHECKS*	INCREASE OF PROTEIN OVER CHECKS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
79	21.8	33.0	21.95	32.80	1.03	-1.27
79A	22.1	32.6				
80	20.7	33.3	21.15	33.85	0.23	-0.22
80A	21.6	34.4				
81	21.2	33.6	21.40	34.05		
81A	21.6	34.5				
82	17.5	39.8	18.10	38.72	-2.82	4.65
82A	18.7	37.6				
83	18.2	43.9	18.10	44.00	-2.82	9.93
83A	18.0	44.1				
84	18.3	43.0	18.30	42.85	-2.62	8.78
84A	18.3	42.7				
85	18.1	44.3	18.05	44.05	-2.87	9.98
85A	18.0	43.8				
86	20.5	34.6	20.10	34.30		
86A	19.7	34.0				
87	17.6	43.8	17.50	44.10	-3.42	9.98
87A	17.4	44.4				
88	18.1	45.2	17.85	45.70	-3.07	11.63
88A	17.6	46.2				
89	21.0	33.5	21.25	33.85		
89A	21.5	34.2				

\* The inoculated, no-lime plots, are used as checks, the same as in table 5.

unlikely that it would pay either to inoculate or to lime the soil alone. The crop yield is so dependent upon both of these factors that neither can be well omitted in the successful growing of soybeans on sour soils.

In general, seed and straw yields follow the same rule as total dry matter, since they, taken together, make up the latter. Bacterial infection in highly

acid soils does not readily take place, as is shown by the small number of nodules on the check plots receiving no lime. All of the plots except 79 to 80A were thoroughly inoculated with well infected soil. Slight infection of plots 80 and 80A was probably due to the transference by wind or animals of a little of the infected soil from plots 81 and 81A. The maximum nodule production took place where there was the greatest amount of lime, namely, in plots 88 and 88A. There is but little difference, however, in the number of nodules per plant on plots receiving small or large applications of lime.

The oil content of soybeans decreases in direct proportion as the application of lime increases in amount; conversely, the protein increases. The oil content of the soybean seeds varied from 17.5 per cent in plot 86 to 21.95 per cent in plot 79. The latter plot had neither lime nor inoculation. The no-lime plots gave an average percentage of oil of 20.92. Taking the results by and large, it is safe to say that liming the soil in this experiment decreased the oil content of the beans about 2.8 per cent. Large applications caused a decrease of over 3 per cent. Plot 80, which was not inoculated but limed, gave practically the same per cent of oil as plot 81, which received no lime but was inoculated. Plot 79, having neither lime nor inoculation, had 1 per cent more oil than these plots. As was found to be the case with crop yield, small amounts of lime were nearly as beneficial as large amounts in increasing the protein content of the soybean seed. The lowest amount of this constituent was found in the check plots where the per cent of oil was highest. Lime without inoculation, or vice versa, is not very efficient in increasing the amount of protein in soybean seeds. Both are necessary.

#### THE YIELD AND COMPOSITION OF SOYBEAN SEEDS AS AFFECTED BY CERTAIN FERTILIZERS AND CHEMICAL SALTS

A hasty review of the literature on this subject reveals the fact that a considerable amount of data has been accumulated on the action of various fertilizers and salts on the protein content of soybeans, but little regarding their action on the oil content.

That fertilizers, especially in the form of phosphorus-containing materials and potash, are quite essential to the production of a maximum crop of soybeans, is the consensus of opinion of the experiment stations of the United States. Soybeans, like most other legumes, draw heavily upon the stores of mineral plant-food in the soil, hence some provision must be made to repay the soil for what it has given to the crop, especially if the fodder or beans are not fed on the farm.

The following experiment stations have recommended applications of fertilizer as follows:

New Jersey (50): 250 pounds acid phosphate; 50 pounds KCl (on light soils).

Rhode Island (2): Applications of acid phosphate, muriate of potash and lime.

Connecticut (21): 200 to 300 pounds of acid phosphate and lime on poor soils.

West Virginia (5): Liberal application of acid phosphate and lime.

Delaware (14): Crimson clover sod supplemented by 250 to 350 pounds of a mixture of 400 pounds of acid phosphate and 100 pounds of muriate of potash.

Ohio (53): Fertilizers not needed on good soils; on poor soils stable manure or complete fertilizers are used with profit.

New York (15): Applications of phosphoric acid and potash if these are deficient in the soil; a small application of nitrate of soda is helpful in giving a vigorous start to the plants.

North Carolina (52): 200 to 400 pounds of acid phosphate and 25 to 50 pounds of muriate of potash. Acid phosphate alone is very beneficial.

Tennessee (35): 300 pounds of acid phosphate, and 250 pounds of wood ashes or 25 pounds of muriate of potash.

Kentucky (40): Organic matter and lime of soil should be increased along with the phosphoric acid.

United States Department of Agriculture (49): Fair application of acid phosphate and KCl on soils of low fertility.

Goessman (13), of Massachusetts, as far back as 1892 claimed that manure and sodium nitrate gave better results with soybeans than minerals alone.

Brooks (4), of Massachusetts, reports that the soybean is one of the crops which responds better to sulfate of potash than to the muriate. He also shows that lime in the different forms increases the yields considerably.

Lipman and his associates (31), in pot experiments with soybeans, showed that there was little difference in the crop yield or nitrogen content of the plants fertilized with various amounts of  $\text{CaSO}_4$ , acid phosphate,  $\text{NaNO}_3$  and  $\text{CaCO}_3$ . The soil used was a loam fairly rich in plant-food, and as the plants were all well inoculated these results were rather to be expected.  $\text{CaCO}_3$  gave as high nitrogen percentages in the plants as did  $\text{NaNO}_3$ .  $\text{CaSO}_4$  gave the lowest percentage of nitrogen in the plants. Doubling the amount of  $\text{CaCO}_3$ ,  $\text{NaNO}_3$  or acid phosphate did not affect the composition (protein content) of the plant appreciably, although the yield was increased.

In another place (33) he showed that gypsum had little or no effect upon the yield or protein content of soybeans. In a liming experiment, 1000 pounds was found to be as beneficial as 4000 pounds. A sandy soil appeared to favor a high protein content of soybeans.

Shive (44) working with soybeans in sand cultures, obtained some interesting results. He found an osmotic concentration of salts of 0.05 to 0.1 atmosphere best for soybeans; greater concentrations were injurious. Ammonium salts, with the exception of  $(\text{NH}_4)_2\text{SO}_4$ , exerted a more toxic effect on soybeans than any of the corresponding salts of K, Na or Ca. Phosphates



caused injury to most of the seedlings grown in solutions containing the higher concentrations of the radical  $\text{PO}_4$ . This may account for the injury to germination which has been repeatedly noted, when soybeans are drilled in direct contact with the fertilizer, the latter usually being acid phosphate.

Lipman and Blair (29) showed that the nitrogen content of soybeans increased with applications of  $\text{NaNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$  and dried blood. In sand cultures they found that nodule development was not depressed by nitrogenous fertilizers. The yield of dry matter increased with the applications of nitrogenous fertilizers up to a maximum, and then decreased. Miss Thompson (46), of the Hawaii Experiment Station, showed that soybeans and other legumes growing in different soil types had varying percentages of nitrogen.

Shedd (43) of the Kentucky Station did much work upon the relation of sulfur to soil fertility. He showed that with soybeans the best results were obtained with sulfur, ammonium sulfate, pyrite and ferrous sulfate. He found that sulfatic fertilizers increased the sulfur content of soybeans, but not necessarily the protein content, as would be expected, since sulfur is a constituent of protein. In two-thirds of the pots, the per cent of protein increases as the sulfur content increases, but not in the same proportion. He showed that ammonium sulfate increased the protein content of soybeans very greatly. In practically all cases sulfur or sulfates gave substantial increases in the dry weight of the soybean plants.

J. K. Wilson (54), in an excellent paper on "Physiological Studies of *Bacillus Radicicola* of Soybeans and of Factors Influencing Nodule Production in Soybeans," points out the effects of a large number of classes of salts on the nodule development. As a general rule chlorides, phosphates, calcium compounds and carbon-containing compounds seemed to stimulate nodule production, while sulfates and ammonia-containing compounds depressed it.

#### *Fertilizers and salts as affecting the oil content of soybean seeds*

The researches of Müntz (37), Leclerc du Sablon (26), Gerber (11, 12), and Ivanov (17) with the poppy, flax, sunflower, rape, soybean, castor bean, hemp and sweet almond, all show that the development of oleaginous seeds is characterized by a progressive accumulation of oil accompanied by a corresponding decrease in carbohydrates. This change takes place in unripe seeds detached from the plant, showing quite conclusively that the oil is derived from carbohydrates. Oil accumulation and protein accumulation progress simultaneously, although there is no evidence that there is any relation between the two processes.

Schulze (42) infers that the plant during the period prior to blooming normally accumulates enough nutrients chiefly in the form of carbohydrates and protein to insure the development of the seed. At or near the blooming stage there begins a general movement of simple sugars and soluble nitrogenous constituents through the stem towards the reproductive parts. In the soybeans the oil is classed as "the nitrogen-free reserve food" of the plant.

Garner, Allard and Foubert (10), of the United States Bureau of Plant Industry, late in 1914 made an admirable contribution to the question of the oil content of seeds as affected by the nutrition of the plant. They studied many phases of the question. Some of the deductions which they make as a result of their work are as follows.

In soybeans except for the period immediately following blooming and that directly preceding final maturity, there is a uniform increase in the oil content, both relative and absolute, throughout the development of the seed, and no evidence was found that there is a critical period of very intense oil formation at any stage of the seed development. Maximum oil production requires conditions of nutrition favorable to carbohydrate accumulation during the vegetative period, and to the transformation of carbohydrate into oil during the reproductive period. There is no correlation between size of seed and oil content. No relation was found to exist between the date of planting soybeans and their oil content at maturity, although some varieties shorten the time required to reach maturity if planted late in season. Different varieties of soybeans vary much in the percentage of oil which they contain. Soil type and climatic influences give variable results with soybeans, but as a general rule, they do not greatly affect the oil content. Climate is a more potent factor in influencing the size of the seed and its oil content than soil type. The relative fertility of the soil appears to be a minor factor in influencing the size of the seed and its oil content. Applications of nitrogenous fertilizers to cotton decreased the oil content; no experiments are reported with the soybean. Cylinder experiments with soybeans fertilized with both phosphatic and potassic minerals gave an increase of 20 per cent in the percentage of oil in the seeds.

With phosphorus alone much the same results were obtained, but potash alone had no effect. Peanuts fertilized with phosphorus or potash did not contain more or less oil than plants not fertilized.

Thus we see the status of the effect of various nutritional factors on the yield and oil and protein content of soybeans.

*Vegetation experiments to determine the influence of soil texture and fertilizer treatment upon the oil content of soybeans*

For this experiment the soils used were Penn fine shaly loam and coarse quartz sand mixed in various proportions as follows:

Pots 1 and 2.	100 per cent Penn fine shaly loam (soil).
Pots 3 and 4.	80 per cent soil and 20 per cent sand.
Pots 5 and 6.	60 per cent soil and 40 per cent sand.
Pots 7 and 8.	40 per cent soil and 60 per cent sand.
Pots 9 and 10.	20 per cent soil and 80 per cent sand.
Pots 11 and 12.	0 per cent soil and 100 per cent sand.

The above is the relative order of the pots in all 8 series. Eight such series of pots were run with various fertilizer treatments. Series I was a check, and no fertilizers were used. Series II was treated with 2 gm. acid phosphate; Series III with 2 gm.  $\text{NaNO}_3$ ; Series IV with 2 gm.  $\text{KCl}$ ; Series V with 2 gm. each of acid phosphate and  $\text{NaNO}_3$ ; Series VI with 2 gm. each of acid phosphate and  $\text{KCl}$ ; Series VII with 2 gm. each of  $\text{NaNO}_3$  and  $\text{KCl}$ , and Series VIII with 2 gm. each of acid phosphate,  $\text{NaNO}_3$  and  $\text{KCl}$ .

The glazed earthenware pots contained 9.5 pounds of soil, or soil and sand as the case might be. Black Eyebrow soybeans were planted in each pot and thinned to 7 plants per pot. The pots were kept at optimum moisture content. This was determined for the first two weeks by weighing the pots, but this entailed so much labor that the practice was discontinued. After weighing a few times it was observed that one could easily tell the amount of water needed to bring the soil to optimum very closely. The crop was not all harvested at the same time since some of the plants matured before others. For instance, the pots of Series III, V and VII matured later than the others, because of the nitrogen with which they were fertilized.

To all of the pots 0.25 gm. of  $\text{MgSO}_4$ , 0.25 gm. of  $\text{Fe}_2(\text{SO}_4)_3$  and 5 gm. of  $\text{CaCO}_3$  were added. All pots were inoculated with soil infusion. The data obtained are given in table 7 (Series I to VIII).

Beginning with table 7, Series I, it is seen that the yield of dry matter in all the pots containing any soil is nearly the same. Pots 3 and 4 are exceptions. Here some unknown harmful factor prevented the plants from growing even after repeated trials. The analysis of the seeds gives but little information of value as to why the pots failed to produce seed. The indications are that a balanced mixture of soil and sand produces higher oil content in the seeds than soil or nearly pure sand. As was to be expected, the pure sand produced but a small crop.

Series II shows an increase in dry matter per pot over Series I. This must be due to the acid phosphate added, as other conditions were the same. The phosphate produced the best results in pots where there was considerable sand mixed with the soil, pointing to a possible better utilization of the phosphorus in sandy soils. As in Series I, the highest oil percentages in the seed are found in soils of medium texture. The general tendency of the results shows that phosphorus slightly increases the oil content of soybean seeds, although the data are not convincing. This may be due to the important rôle phosphorus is supposed to play in seed formation.

The addition of  $\text{NaNO}_3$  in Series III causes a decrease in both dry-matter and oil content of seeds. This is in keeping with the results of other experiments herein reported. The oil content is decreased from 1 to 2 per cent in nearly all of the pots. The effect of the nitrate was further pointed out by the tall, spindling, weak vines produced by the plants in this series. In the pure sand pots of both Series II and III there is but little plant growth, showing that essential elements are lacking there.

TABLE 7  
*Effect of fertilizers upon the oil content of soybeans*

Series I. No fertilizers

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	<i>gm.</i>	<i>gm.</i>	<i>per cent</i>
1	12.0	12.05	18.2
2	12.0		
3*	2.3	1.65	No seed
4*	1.0		
5	9.4	11.10	19.0
6	12.8		
7	12.0	12.60	19.3
8	13.2		
9	11.3	11.85	17.9
10	12.4		
11	6.9	6.85	No seed
12	6.8		

Series II. 2 gm. acid phosphate

13	12.2	12.50	18.7
14	12.8		
15	12.1	13.60	19.0
16	15.1		
17	14.2	13.50	19.5
18	12.2		
19	13.3	14.90	18.6
20	16.5		
21	10.2	9.55	Too few seeds
22	8.9		
23	7.1	6.65	No seed
24	5.2		

\* Some unknown harmful factor caused the plants to die even after repeated plantings.

TABLE 7—*Continued*  
 Series III. 2 gm.  $\text{NaNO}_3$  per pot

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	<i>gm.</i>	<i>gm.</i>	<i>per cent</i>
25	9.4	12.05	17.2
26	14.7		
27	12.6	11.95	17.3
28	11.3		
29	16.0	13.85	17.6
30	11.7		
31	13.3	13.30	18.1
32	13.3		
33	12.0	12.55	18.0
34	13.1		
35	6.0	6.15	No seed
36	6.3		

Series IV. 2 gm.  $\text{KCl}$  per pot

37	13.8	13.30	18.1
38	12.8		
39	12.1	11.95	18.5
40	11.8		
41	7.4	8.05	15.3†
42	8.7		
43	9.8	10.50	17.6†
44	11.2		
45	6.3	7.05	Too little seed
46	7.8		
47	3.0	3.85	No seed
48	4.7		

† Small seeds.

TABLE 7—*Continued*Series V. 2 gm. acid phosphate + 2 gm. NaNO<sub>3</sub>

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	<i>gm.</i>	<i>gm.</i>	<i>per cent</i>
49	16.4	15.15	17.8
50	14.9		
51	15.0	15.30	17.7
52	15.6		
53	15.7	16.10	18.0
54	16.5		
55	16.0	15.90	18.3
56	15.8		
57	13.7	12.75	17.4
58	11.8		
59	8.5	8.25	Too few seeds
60	8.0		

Series VI. 2 gm. acid phosphate + 2 gm. KCl

61	13.3	11.50	18.6
62	9.7		
63	12.8	11.35	18.1
64	9.9		
65	12.0	12.00	18.2
66	12.0		
67	9.3	8.75	16.7†
68	8.2		
69	6.8	6.05	No seeds
70	5.3		
71	3.0	3.45	No seeds
72	3.9		

TABLE 7—*Continued*  
 Series VII. 2 gm.  $\text{NaNO}_3$  and 2 gm. KCl

POT NO.	DRY MATTER	AVERAGE DRY MATTER	OIL IN SEEDS
	<i>gm.</i>	<i>gm.</i>	<i>per cent</i>
73	12.1	11.55	16.7†
74	11.0		
75	11.7	11.70	17.6†
76			
77	14.4	13.45	17.8
78	12.5		
79	11.4	11.35	17.2†
80	11.3		
81	8.5	7.75	No seed
82	7.0		
83	6.0	5.50	No seed
84	5.0		

Series VIII. 2 gm. acid phosphate, 2 gm.  $\text{NaNO}_3$  and 2 gm. KCl

85	14.0	14.00	18.10
86	14.0		
87	14.0	14.45	18.30
88	14.9		
89	12.8	12.60	18.40
90	12.4		
91	14.5	13.60	17.60†
92	12.7		
93	9.8	9.40	16.30†
94	9.0		
95	2.6	3.20	No seed
96	3.8		

† Small seeds.

The results of Series IV fertilized with KCl alone are much the same as those of the check Series I, only the yield of dry matter in several of the pots is even less than in the latter series. The per cent of oil in the seeds is variable, but seems to be a little lower in the pots fertilized with potash alone than in Series I and II, where there were no fertilization and 2 gm. of acid phosphate, respectively. The oil content, however, was higher than in Series III, fertilized with  $\text{NaNO}_3$ .

Series V produced the largest yield per pot of any series thus far. The oil content is intermediate between Series II and Series III, as would be expected since both acid phosphate and nitrate of soda are added to each pot in this series.

In Series VI, fertilized with KCl and acid phosphate, the yield of dry matter is lower than in Series II, having acid phosphate alone. Such results cannot be accounted for unless KCl tends to depress the yield, as it apparently did in Series IV. The percentage of oil increases again over Series V, as no  $\text{NaNO}_3$  is present, and compares favorably with Series II, where only acid phosphate was used. It was noticed that, in some of the pots of all of the series, small seeds occurred in some of the pods. This may have been due to lack of proper nourishment or other causes, but these small seeds always gave a lower percentage of oil than the large well-formed seeds. Oil determinations made from plants bearing many small seeds are marked with a star to call attention to the fact. Such determinations were invariably low.

Series VII, fertilized with both  $\text{NaNO}_3$  and KCl, gave yields of dry matter slightly lower than Series III, which had  $\text{NaNO}_3$  only. The oil percentages also are low, attributable no doubt to the soluble nitrogenous salt.

Complete fertilizer was used on Series VIII with the result that good healthy-looking plants were obtained, although the yield of the series as a whole was not as high as of Series V, containing acid phosphate and  $\text{NaNO}_3$  only. The yield is higher than from any of the other series, however. The oil content also is fairly high but not as high as in a number of other series. Where a complete fertilizer is present the peculiar effect of  $\text{NaNO}_3$  in lowering the percentage of oil in the seed does not seem to be as noticeable.

#### *Deductions from the vegetation experiments*

No practical deductions may be drawn from these vegetation experiments because the results obtained are not sufficiently conclusive, and also because of the variability of the percentage of oil in soybean seeds, obtained from different pots.  $\text{NaNO}_3$  decreased the oil content of the seeds in every case. Acid phosphate generally increased it slightly or else had no effect. KCl appeared to depress the oil content slightly. Too great concentrations of salts in the sand or near sand pots may account for the stunted plant growth obtained in most of these pots.



After harvesting the crop the soil was well stirred up, and a second crop of soybeans planted. The red spider attacked the young plants with such avidity that the experiment was ruined and discontinued.

*Field experiments conducted to determine the effect of various fertilizers upon the yield, nodule production, oil and protein content of soybeans*

The plots used in this experiment were located on Penn fine sandy loam. They were 5 by 10 feet in size, comprising 0.001141 acre. The soil was very uniform in fertility as shown by the previous grass crop. All plots were divided from each other by a space of 1 foot. The inoculation used was soil from an old soybean field, applied at the rate of 2 pounds per plot. The infection was very good. A peculiar yet very important observation was made concerning the spread of the nodule-producing bacteria in the soil. Many soybean plants were growing outside of the plots so as to make the end plots of the experiment as nearly as possible like the others, and it was noticed that these plants had either very few nodules or no nodules at all, even though they were growing within a few feet of richly infected soil. The plots were planted to Black Eyebrow soybeans on June 4, 1916, and harvested on September 19, 107 days later. The beans were planted in rows, three rows per plot, and carefully cultivated during the summer. To determine the dry matter produced on each plot, samples of ten plants were carefully weighed, dried, and weighed again. It was then a simple matter to get the dry weight of the total yield of each plot. Of course, the material on each plot was weighed green as harvested, and later again when the beans were flailed out to determine the relative yields of straw and seed. The lime used was ground oyster shells. The lime requirement of the soil as shown by the Veitch method was about 6000 pounds of CaO. The amount of  $\text{CaCO}_3$  used on the limed plots was 2000 pounds per acre. Various fertilizers and combinations of fertilizers, both with and without lime were used.

The results are given in the following tables.

Table 8 shows that applications of acid phosphate to soybeans give increased yields, provided plenty of lime is present in the soil. Small applications 100 to 200 pounds per acre were as beneficial as greater amounts on limed soil. The yield of seed also was increased by the phosphatic fertilizer. From these experiments it appears that small applications of 100 to 200 pounds of acid phosphate per acre may be profitably used on limed soils, but not on unlimed soils. On acid soils lime is apparently a greater factor than plant-food. It corrects the soil acidity, mellows heavy soils, makes the plant-food in the soil more available to plants by stimulating bacterial activity, and lastly, makes the soil a congenial home for the nitrogen-gathering bacteria. Nodule formation was increased considerably on the limed plots by the acid phosphate, especially in the plots receiving from 100 to 300 pounds per acre; on the unlimed plots there was little or no increase in the

number of nodules per plant where phosphorus was applied. Comparing the limed with the unlimed plots it is seen at a glance that both yield of dry matter and bean seed, and the number of nodules per plant were much increased by the use of lime with the acid phosphate.

TABLE 8  
*Effect of phosphates on yield and nodule production in soybeans*

PLOT	TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	
1	2000 pounds CaCO <sub>3</sub>	4.90	2.15	2.75	4.93	2.16	0.36	0.30	20.2
1A	100 pounds acid phosphate	4.95	2.16	2.79					
2	2000 pounds CaCO <sub>3</sub>	5.06	2.06	3.00	4.95	1.98	0.38	0.12	24.8
2A	200 pounds acid phosphate	4.83	1.90	2.93					
3	2000 pounds CaCO <sub>3</sub>	4.92	1.74	3.18	4.80	1.76	0.27	-0.10	26.4
3A	300 pounds acid phosphate	4.67	1.77	2.90					
4	2000 pounds CaCO <sub>3</sub>	4.96	2.00	2.96	4.75	1.91	0.18	0.05	18.5
4A	500 pounds acid phosphate	4.53	1.82	2.71					
5	Check	4.59	1.81	2.68	4.57	1.86			18.6
5A	2000 pounds CaCO <sub>3</sub>	4.54	1.91	2.63					
6	No lime	4.34	1.57	2.77	4.12	1.59	0.24	0.23	17.4
6A	100 pounds acid phosphate	3.89	1.61	2.28					
7	No lime	3.63	1.32	2.31	3.67	1.39	-0.21	0.03	21.3
7A	200 pounds acid phosphate	3.70	1.45	2.25					
8	No lime	3.54	1.12	2.42	3.47	1.26	-0.41	-0.10	13.1
8A	300 pounds acid phosphate	3.40	1.37	2.03					
9	No lime	3.42	1.10	2.32	3.43	1.18	-0.45	-0.18	14.6
9A	500 pounds acid phosphate	3.43	1.25	2.18					
10	Check	3.93	1.43	2.40	3.88	1.36			14.8
10A	No lime, no acid phosphate	3.83	1.29	2.64					

The use of acid phosphate with lime seemed to cause a small yet consistent increase in the oil content of beans over the check plot (table 9). In the case of the unlimed plots phosphorus did not raise the percentage of oil in the seed. As regards the protein content, little or no influence of phos-

phorus is noted on the limed plots, but on the unlimed there seems to be a small increase in the percentage of protein in the seed due to the application of acid phosphate. Since phosphorus is a constituent of protein, one would rather expect heavy applications of phosphatic fertilizers to increase the percentage of the former in the seed, but this does not necessarily hold true, since many other limiting factors may enter into the problem.

TABLE 9  
*Effect of phosphates on the oil and protein content of soybeans*

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	17.8	42.5	17.50	42.60	1.26	-0.05
1A	17.2	42.7				
2	16.9	42.0	17.00	42.00	0.75	-0.65
2A	17.1	42.0				
3	17.6	41.5	16.85	42.40	0.60	-0.25
3A	16.1	43.3				
4	16.3	43.3	16.30	42.55	0.05	-0.10
4A	16.3	41.8				
5	16.6	42.6	16.25	42.65		
5A	15.9	42.7				
6	18.1	39.2	17.90	39.60	-1.25	1.30
6A	17.7	40.0				
7	19.4	39.0	19.40	38.40	0.75	0.10
7A	19.4	37.8				
8	17.8	38.6	18.15	39.45	-0.50	1.15
8A	18.5	40.3				
9	17.9	38.9	18.60	39.25	-0.05	0.95
9A	19.3	39.6				
10	18.6	38.0	18.65	38.30		
10A	18.7	38.6				

Potash, although an essential element to plant growth, does not seem to be needed as much on most soils as phosphorus or nitrogen. On the Penn loam, however, where this series of experiments was conducted, it gave increased yields of dry matter and seed over the checks, in all applications from 50 to 400 pounds per acre (table 10). This increased yield took place on both limed and unlimed plots. The increase was approximately 10 per cent,

hence from a practical point of view it would pay to make a small application of potash to soybeans. Small applications gave practically the same results, or even better results than higher amounts. Potash stimulated

TABLE 10

*Effect of potash on the yield and nodule production of soybeans*

PLOT NO.	FERTILIZER TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK	INCREASE IN SEED OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
11	50 pounds KCl	4.89	2.00	2.89	4.86	1.98	0.53	0.10	25.4
11A	2000 pounds CaCO <sub>3</sub>	4.83	1.95	2.88					
12	100 pounds KCl	4.84	2.04	2.80	4.75	2.00	0.52	0.12	25.7
12A	2000 pounds CaCO <sub>3</sub>	4.66	1.97	2.69					
13	200 pounds KCl	4.40	1.95	2.45	4.52	1.93	0.19	0.05	23.8
13A	2000 pounds CaCO <sub>3</sub>	4.65	1.90	2.75					
14	400 pounds KCl	4.33	2.05	2.28	4.64	2.09	0.31	0.11	31.4
14A	2000 pounds CaCO <sub>3</sub>	4.94	2.13	2.81					
15	Check	3.88	1.86	2.02	4.33	1.88			21.2
15A	2000 pounds CaCO <sub>3</sub>	4.77	1.90	2.87					
16	50 pounds KCl	4.91	1.80	3.11	4.87	1.85	0.87	0.19	19.0
16A		4.83	1.90	2.93					
17	100 pounds KCl	4.68	2.00	2.68	4.63	1.96	0.63	0.30	20.2
17A		4.58	1.93	2.55					
18A	200 pounds KCl	4.08	1.56	2.52	4.12	1.60	0.12	-0.06	27.9
18A		4.16	1.64	2.46					
19	400 pounds KCl	4.08	1.65	2.43	4.20	1.68	0.20	0.02	16.9
19A		4.31	1.71	2.60					
20	Check	3.99	1.71	2.28	4.00	1.66			20.9
20A		4.02	1.60	2.42					

nodule production slightly on the limed plots, but no differences were obtained on the unlimed plots. Here again the yield of dry matter and seeds was considerably greater on the limed plots.

Potash appears to decrease slightly the oil content of soybean seeds in all the plots except one (table 11). The same is true of the percentage of pro-

tein, although the differences are almost negligible. On the limed plots the oil content is considerably lower than on the unlimed plots.

For calculating the increase in yield over the check in this experiment it was deemed better to use as the check the average of 6 plots, namely, 5 and 5A, 15, 15A, 28 and 28A than to use only plots 28 and 28A, included prop-

TABLE 11  
*Effect of polish on the oil and protein content of soybeans*

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
11	17.5	39.7	17.0	39.0	-1.8	-4.4
11A	16.5	40.8				
12	17.5	43.0	17.9	43.3	-0.9	-0.1
12A	18.2	43.5				
13	17.5	42.0	17.7	41.3	-1.1	-2.1
13A	17.9	40.5				
14	15.7	41.4	15.9	41.3	-2.9	-2.1
14A	16.0	41.1				
15	17.4	43.0	18.8	43.4		
15A	20.1	43.8				
16	18.7	39.9	18.7	39.7	-0.63	-0.0
16A	17.6	39.4				
17	18.4	39.2	17.8	39.7	-1.1	0.0
17A	17.3	40.2				
18	19.8	38.7	19.6	39.0	-1.7	-0.7
18A	19.3	39.2				
19	18.5	38.4	18.1	38.8	-0.8	-0.9
19A	19.6	39.2				
20	18.3	39.8	18.9	39.7		
20A	19.4	39.6				

erly in this experiment. As the soil is very uniform it is permissible to do this, and thus secure more reliable data. All of the plots received 2000 pounds of ground oyster shells in addition to the fertilizer mixtures as given in the tables. Because of an error in fertilizing, no check plot was left at plots 24 and 24A, where one would naturally have occurred according to the original scheme of the experiment.

All of the fertilizer mixtures gave increases over the check plots in the yield of dry matter, and except for plots 27 and 27X, treated with manganese, in seed also (table 12). The most efficient combination of fertilizers seemed to be that combining nitrogen, phosphorus and potash. These combinations make up a complete fertilizer, and gave the largest increases over the check, as a general thing. Whether such a fertilizer is economical or not is a different matter. It is likely that the greatest returns for money invested would be obtained by using lime and acid phosphate alone. The fact that plot 23 had 200 pounds per acre more potash than plot 22 does not seem to affect the yield favorably.

However, plot 21, with only 100 pounds per acre of potash, gives as great a yield as plot 23, with 400 pounds. From these results it would seem to indicate that small applications of potash up to 100 pounds per acre might pay on soils like the one where the experiment was carried on. Nitrate of soda produced increased dry matter and seed yields, showing that where soluble nitrogenous plant-food is readily accessible to the soybean plant it is absorbed and used by the latter. This is not an economical way of supplying plant-food, however, since it has been demonstrated that legumes are able to obtain the greater part of their nitrogen from the atmosphere. The presence of 200 pounds per acre of nitrate of soda very seriously interferes with nodule formation, as is shown by plot 26. This plot produced plants having on an average 15.2 nodules, while the check plot produced about 26. Nodule formation was not notably depressed by the other combination of fertilizer used. Wherever nitrate was used a slight reduction in the number of nodules per plant was noticed. As shown before, phosphorus and potash have a stimulating action upon nodule formation.

The use of manganese sulfate as a fertilizer or chemical stimulant has been advocated by many men, and has been used in this way on various crops with widely different results. As much manganese ore refuse is easily obtained at the zinc mines in northern New Jersey, it was decided to try its effect upon the growth of soybeans. The data show that a benefit is derived from the use of 50, 100 and 500 pounds per acre of  $\text{MnSO}_4$ . The yield of seed is not increased except on plot 31 with an application of 500 pounds per acre. The increases are rather small, so it is not likely that it would pay to apply manganese-containing substances as a fertilizer. Besides, the beneficial results obtained from the use of  $\text{MnSO}_4$  may come from the  $\text{SO}_4$  radical and not from the Mn. That it does stimulate germination and early growth of soybeans is apparently true, as the manganese-treated plots were up a number of days before any of the others. The early growth was also stimulated but the other plots soon equaled the manganese-treated plots in height, and many passed them within two weeks from the date of planting. There is little to indicate that nodule production is greatly affected by applications of manganese salts; if anything they have a slightly depressing effect.

TABLE 12

*Effect of various fertilizers on the yield and nodule production of soybeans*

PLOT NO.	FERTILIZER TREATMENT	TOTAL DRY MATTER	SEED	STRAW	AVERAGE TOTAL DRY MATTER	AVERAGE SEED	INCREASE IN TOTAL DRY MATTER OVER CHECK†	INCREASE IN SEED OVER CHECK†	NUMBER OF NODULES PER PLANT
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
21	Acid phosphate 300 pounds KCl 100 pounds	5.23	2.08	3.15	5.43	2.09	0.92	0.23	28.1
21A	CaCO <sub>3</sub> 2000 pounds	5.63	2.10	3.53					
22	Acid phosphate 300 pounds KCl 200 pounds	4.16*	1.90	2.26	4.71	1.95	0.20	0.09	28.7
22A	CaCO <sub>3</sub> 2000 pounds	5.25	1.99	3.26					
23	Acid phosphate 300 pounds KCl 400 pounds	5.41	2.06	3.35	5.41	2.08	0.90	0.22	34.9
23A	CaCO <sub>3</sub> 2000 pounds	5.40	2.09	3.31					
24	Acid phosphate 300 pounds CaCO <sub>3</sub> 2000 pounds	5.88	2.12	3.76	6.36	2.14	1.85	0.28	26.6
24A	KCl none	6.84	2.17	4.67					
25	Acid phosphate 300 pounds KCl 200 pounds	6.03	2.24	3.79	6.11	2.20	1.60	0.34	24.7
25A	CaCO <sub>3</sub> 2000 pounds NaNO <sub>3</sub> 100 pounds	6.18	2.16	4.02					
26	Acid phosphate 300 pounds KCl 200 pounds	6.22	2.34	3.88	6.25	2.32	1.74	0.46	15.2
26A	CaCO <sub>3</sub> 2000 pounds NaNO <sub>3</sub> 200 pounds	6.27	2.29	3.98					
27	MnSO <sub>4</sub> 100 pounds	5.03	1.45	3.58	4.97	1.70	0.46	-0.14	23.5
27A	CaCO <sub>3</sub> 2000 pounds	4.90	1.94	2.96					
27X	MnSO <sub>4</sub> 50 pounds	4.52	1.62	2.90	4.71	1.82	0.20	-0.04	23.5
27AX	CaCO <sub>3</sub> 2000 pounds	5.06	2.02	3.04					
28	Check	4.14	1.67	2.47	4.63	1.84			26.1
28A	CaCO <sub>3</sub> 2000 pounds	5.13	2.00	3.23					
29	KCl 200 pounds NaNO <sub>3</sub> 200 pounds	4.53	1.85	2.68	4.63	1.98	0.12	0.12	25.9
29A	CaCO <sub>3</sub> 2000 pounds	4.72	2.10	2.72					
30	KCl none	4.83	1.87	2.96	5.51	2.02	1.00	0.16	23.8
30A	NaNO <sub>3</sub> 200 pounds CaCO <sub>3</sub> 2000 pounds	6.18	2.16	4.02					
31	MnSO <sub>4</sub> 500 pounds CaCO <sub>3</sub> 2000 pounds	5.00	2.11	2.89	5.00	2.11	0.49	0.25	

\* Sample for moisture determination partly eaten by rats.

† The average value of check plots No. 5, No. 15 and No. 28 is meant here.

In regard to the effect of fertilizer mixtures upon the oil and protein content of soybean seeds, the data are conflicting and not convincing (table 13).  $MnSO_4$  in all three plots greatly depressed the percentage of protein in the beans, and two of the three plots gave an increase in oil content over the check.

TABLE 13  
*Effect of various fertilizers on the oil and protein content of soybeans*

PLOT NO.	OIL	PROTEIN	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
21	19.9	43.5	19.65	43.65	1.68	0.82
21A	19.4	43.8				
22	16.5	43.8	16.10	43.40	-1.87	0.57
22A	15.7	43.0				
23	18.7	42.8	19.00	43.05	0.65	0.22
23A	19.3	43.3				
24	17.2	43.5	17.15	43.40	-0.82	0.57
24A	17.1	43.3				
25	17.4	45.3	18.15	44.75	0.18	1.92
25A	18.9	44.2				
26	19.0	44.0	18.50	43.65	0.53	0.82
26A	18.0	43.3				
27	18.3	40.1	18.15	40.45	0.18	-2.38
27A	18.0	40.0				
27X	17.8	41.5	18.55	42.25	-0.58	-0.58
27AX	19.3	43.0				
28	19.1	42.0	18.85	42.45		
28A	18.6	42.9				
29	17.9	42.0	17.35	42.65	-0.62	-0.38
29A	16.8	43.3				
30	18.9	41.4	18.9	42.30	0.93	-0.53
30A	18.9	43.2				
31	19.4	41.1	19.4	41.10	1.43	-1.73

Phosphorus and potash, as in other experiments herein reported, appear to increase the protein content slightly, but the results with the oil are too variable to warrant the drawing of even a tentative conclusion. Possibly one reason for the failure to obtain greater differences in crop yield and com-



position was the fact that all the plots were limed, thus liberating sufficient plant-food for the crop's needs, as well as stimulating the nodule bacteria and a greater fixation of nitrogen.

*Field experiments on the effect of sulfur, sulfates, and nitrates upon the yield, oil, and protein content of soybeans*

For this experiment plots were laid out on acid Sassafras sandy loam on land adjoining the lime and inoculation experiments already discussed. The plots were laid out exactly the same as in previous experiments, except for the fertilizer treatment. This consisted of Basic slag, 400 pounds per acre; muriate of potash, 200 pounds; and ground oyster shells, 2000 pounds. Besides this general treatment the plots were fertilized with special applications of sulfur, sulfates and nitrates. All of the plots were inoculated by spreading about 2 pounds of inoculated soil on each plot. Black Eyebrow soybeans were planted July 1 and harvested on October 2. The A plots were of slightly higher fertility than the duplicates, as the result of a heavier sod in places, but the soil as a general thing was fairly uniform. The native vegetation on the soil previous to plowing showed it to be of poor fertility. The results obtained are given in tables 14 and 15.

*Elemental sulfur* did not give increased yields of seed in these experiments in quantities over 200 pounds per acre. An application of 200 pounds per acre resulted in a slightly increased yield of seed, but there is no doubt that on this soil, greater amounts are injurious to soybeans. The yield of dry matter was noticeably lower on the plots having applications of 400 and 600 pounds per acre than on the check plots. Unfortunately, these data were lost and we have only the yield of seed as a criterion.

From the data it appears that the protein content of the seeds is increased by a moderate application of sulfur, but decreased by larger amounts. That sulfur in quantities over 200 pounds per acre interfered with the normal growth of the plants was readily observed throughout the experiment. The plants were not healthy or leafy, but seemed to produce considerable seed in spite of this. Since sulfur is a constituent of protein it seems reasonable that moderate amounts would increase the percentage of protein in the seeds. This was found to be the tendency of the results obtained by Shedd (43), of the Kentucky Experiment Station. However, he analyzed the immature plants and not the seeds.

The oil content of soybean seeds, contrary to the results obtained for protein, is decreased by moderate applications of sulfur and increased by the larger ones. In the seed obtained from plot 102, where sulfur at the rate of 400 pounds per acre was applied, the increase in the content of oil was 1.4 per cent.

Nodule production is probably somewhat stimulated by small applications of sulfur. The data are not conclusive, because of the wide variation between checks.

*Calcium sulfate* or gypsum was found to exert little influence on the yield of seed until 600 pounds per acre had been applied, when a small increase in the seed yield was noted. As in the case with free sulfur, the oil content was raised and the protein content of the seeds lowered, with large applications (600 pounds) of land plaster per acre. Calcium sulfate caused an ap-

TABLE 14  
*Effect of sulfur and gypsum on the yield and composition of soybeans*

PLOT NO.	FERTILIZER TREATMENT	SEED	OIL	PROTEIN	AVERAGE SEED	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN SEED OVER CHECK	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	per cent	per cent	lbs.	per cent	per cent	lbs.	per cent	per cent	
100	Check	0.87	19.5	40.3	0.98	19.8	39.5				4.8
100A		1.08	20.2	38.6							
101	200 pounds sulfur	1.03	16.8	40.2	1.08	17.4	40.3	+0.15	-0.80	+0.6	7.7
101A		1.12	17.8	40.4							
102	400 pounds sulfur	0.87	19.9	40.3	0.88	19.6	39.6	-0.05	+1.40	-0.1	11.6
102A		0.88	19.2	38.8							
103	600 pounds sulfur	0.71	18.0	36.1	0.73	18.4	43.4	-0.20	+0.2	-2.3	6.0
103A		0.74	18.7	38.6							
104	Check	0.89	16.3	40.9	0.88	16.5	39.8				11.2
104A		0.86	16.6	38.7							
105	200 pounds CaSO <sub>4</sub>	0.87	17.3	41.7	0.87	17.4	40.9	-0.17	-1.1	+0.2	15.4
105A		0.87	17.5	40.0							
106	400 pounds CaSO <sub>4</sub>	0.98	17.2	40.3	0.98	17.5	38.9	-0.06	-1.0	-1.8	19.7
106A		0.98	17.8	37.6							
107	600 pounds CaSO <sub>4</sub>	1.27	19.3	38.6	1.34	19.7	38.6	+0.30	+1.2	-2.1	20.9
107A		1.41	20.1	38.6							
108	Check	1.17	20.1	42.0	1.19	20.4	41.5				12.9
108A		1.20	20.7	41.0							

parent increase in nodule production in all the plots where it was used. This is not in accord with the work of Wilson (54), of Cornell, who found it depressed nodule formation in young soybean plants. He found also that MnSO<sub>4</sub> stimulated nodule production, while ZnSO<sub>4</sub> and iron tersulfate depressed it. In the present experiment it was found that, if anything, MnSO<sub>4</sub> slightly depressed the number of nodules per plant, while ZnSO<sub>4</sub> and F<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> caused a

still greater depression. All of the sodium-nitrate-fertilized plots caused a very marked decrease in the number of nodules per plant.

Concerning the yield of seed on the  $\text{MnSO}_4$ ,  $\text{ZnSO}_4$ , and  $\text{Fe}_2(\text{SO}_4)_3$  plots the data show a slight increase for the latter two plots, but for the plots

TABLE 15  
*Effect of sulfates and nitrates on yield and composition of soybeans*

PLOT NO.	FERTILIZER TREATMENT	SEED	OIL	PROTEIN	AVERAGE SEED	AVERAGE OIL	AVERAGE PROTEIN	INCREASE IN SEED OVER CHECK	INCREASE IN OIL OVER CHECK	INCREASE IN PROTEIN OVER CHECK	NUMBER OF NODULES PER PLANT
		lbs.	per cent	per cent	lbs.	per cent	per cent	per cent	per cent	per cent	
109	$\text{NaNO}_3$	1.39	19.7	40.6	1.29	20.2	40.0	0.08	+0.8	-0.7	20.7
109A	100 pounds	1.19	20.7	39.9							
110	$\text{NaNO}_3$	1.57	20.8	39.6	1.58	20.8	39.8	+0.21	+1.4	-0.9	18.5
110A	200 pounds	1.58	20.7	39.6							
111	$\text{NaNO}_3$	1.60	18.4	43.3	1.58	18.2	43.3	+0.21	-1.2	+2.6	16.0
111A	600 pounds	1.56	18.0	43.3							
112	Check	1.53	18.1	40.3	1.55	18.3	39.9				28.3
112A		1.56	18.4	39.5							
113	$\text{NaNO}_3$	1.37	18.3	42.4	1.52	18.7	42.2	-0.19	0.0	+1.4	18.3
113A	400 pounds	1.67	19.1	42.0							
114	$\text{MnSO}_4$	1.43	19.2	39.9	1.62	19.8	39.6	-0.09	+1.1	-1.2	16.0
114A	50 pounds	1.81	20.3	39.2							
115	$\text{MnSO}_4$	1.17	16.7	40.1	1.26	17.3	40.6	-0.45	-1.4	-0.2	24.6
115A	100 pounds	1.35	17.8	41.0							
116	$\text{ZnSO}_4$	1.54	18.0	42.7	1.37	18.2	42.6	+0.16	-0.5	+2.2	21.2
116A	100 pounds	2.20	18.3	42.4							
117	Check	1.76	19.0	41.0	1.86	19.1	41.8				27.0
117A		1.96	19.2	42.5							
118	$\text{FeSO}_4$	2.54	18.4	41.3	2.54	18.4	41.8	+0.68	-0.7	0.0	16.2
118A	100 pounds	2.54	18.4	42.3							

fertilized with 50 and 100 pounds of  $\text{MnSO}_4$  per acre there was a decrease. This is in accord with other experiments reported in this paper, where  $\text{MnSO}_4$  caused a small decrease in drop yield on Penn loam soil; zinc and iron sulfates slightly increased the oil content of the soybeans produced on these plots, the former causing an increase of 2.2 per cent in protein over checks

and the latter no increase or decrease over checks.  $MnSO_4$  increased the protein content of beans from both plots where it was used, but the oil content is increased by an application of 50 pounds per acre and decreased by an application of double this amount. Such results mean nothing, and conclusions drawn from them, unless the experiments have been repeated a number of times, are worthless.

*Sodium nitrate* gave variable results in regard to the yield of seed. It is certain, however, that it would not pay to use it as a fertilizer for soybeans on this type of soil. Large applications, 400 and 600 pounds per acre, increased the protein content of the seeds appreciably, but the use of smaller amounts did not. Small amounts of nitrate on this soil gave an actual increase in oil content of the soybeans, but an application of 400 pounds per acre gave no increase, and one of 600 pounds per acre gave a decrease of 1.2 per cent.

#### SUMMARY AND PRACTICAL DEDUCTIONS

From both vegetation and field experiments, it was found that certain commercial cultures of bacteria for inoculating soybeans are not reliable, while others are as efficient in producing nodules in the host plant as freshly-isolated cultures of *B. radicicola* or well infected soil. Inoculation gave a substantial increase in the yield of total dry matter and of seed of soybeans in every case. An average decrease of 3 per cent in the oil content of soybean seeds was caused by inoculation. The protein content, on the contrary, was increased 7 per cent. The oil content is decreased and the protein content increased in direct proportion to the thoroughness of infection of the plants. No differences in the drying power of the oil extracted from the seeds of inoculated and uninoculated plants was observed. Natural inoculation of soil sometimes spreads very slowly, as it was repeatedly observed that plants on inoculated plats at a distance of a foot or two from uninoculated plats, were seldom found inoculated. It appears that unless *B. radicicola* is transferred by means of winds, water, animals, etc., its progress is very slow in the soil.

Ground oyster shells and burnt lime were very efficient in increasing the yield and total dry matter of soybeans on acid soils; the increase varying from 30 to 50 per cent. Small applications (1000 to 2000 pounds per acre) are nearly as beneficial as large amounts, and are, of course, much more economical. Small applications of lime at intervals of a few years are to be preferred to a single large application. It appears that liming soybeans on acid soils is nearly as important as inoculation. Both should be practised together for the best results. On sour soils liming stimulated nodule production to a marked degree—in some cases as much as 1500 per cent. The bacterial infection of roots does not take place readily on acid soils even when the root-infecting organisms are plentiful in the soil. The oil content of soybeans decreases in direct proportion to the largeness of applications of lime

applied to the soil; conversely, the protein increases. The average decrease in oil content due to liming was 2.8 per cent. Small amounts of lime are nearly as efficient in raising the protein content of soybean seeds as larger applications.

Immature and small seeds are lower in oil content than mature seeds. This may be explained by assuming that reserve carbohydrates in the seed have not become fully transformed into oil.

The yield of total dry matter and seed of soybeans is materially increased by small applications of acid phosphate, especially when the soils are well limed. One to two hundred pounds per acre appears to be as beneficial as large applications. On acid soils, acid phosphate did not give any appreciable increase, hence the soil should be first limed before applying phosphatic fertilizers. Nodule production on soybeans was also stimulated on limed soils by acid phosphate but this was not so marked on acid soils. Oil production in the seeds was increased on the limed plots but not on the unlimed. Acid phosphate, however, seems to exert a beneficial influence on protein formation in the seed on both limed and unlimed plots.

Potash (muriate), in applications of from 50 to 400 pounds per acre, gave an average increase of about 10 per cent in the yield of total dry matter and seed on both limed and unlimed plots. Nodule production was slightly stimulated on the limed plots, but not on the unlimed. Potash caused a slight decrease in the percentage of oil in the seeds, but had little influence on their protein content.

Various combinations of acid phosphate, muriate of potash and nitrate of soda, with a dressing of lime, all gave substantial increases in the yield of total dry matter and except for two plots fertilized with manganese sulfate, in seed as well. That fertilizer treatment which would appear to give the greatest return for the money invested on acid soils, with soybeans as the crop, is probably 200 to 300 pounds of acid phosphate together with a ton of lime. Other fertilizer mixtures give increases in the crop, but they are not sufficient to justify using them. Nitrate of soda, for example, increased the yield but inhibited nodule formation and consequent fixation of atmospheric nitrogen. It is not economical to supply soluble plant-food in the form of nitrogenous fertilizers to soybeans. Nitrate of soda caused an appreciable increase in the protein content of soybean seeds, and also a decrease in their oil content.

Manganese sulfate stimulated germination and early growth of soybeans but did not stimulate nodule production nor give increased yields. It had little, if any, effect upon the oil or protein content of the seed.

Elemental sulfur did not give increased yields of dry matter or seed in applications over 100 pounds per acre. Larger amounts seemed to injure the plants. Perhaps this was due to the oxidation of the sulfur in the soil to  $H_2SO_4$ , thus producing acidity. It appears that the protein content of soybeans is increased by moderate applications of sulfur, but is decreased by

large applications. The exact reverse is true in the case of oil content. In general, sulfur stimulates nodule formation.

Land plaster in amounts up to 600 pounds per acre exerted little influence on the yield of total dry matter or seed. Large amounts caused an increase in oil content in the seed and also stimulated nodule formation.

The results on the plots where zinc sulfate and ferric sulfate were used are not conclusive, but these minerals seemed to stimulate the growth of the plants and gave increased seed production. The protein content also was somewhat increased. The oil content was slightly decreased.

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**PLATE 1**

**EFFECT OF INOCULATION ON THE SIZE AND LEAFINESS OF SOYBEANS**



PLATE 2

EFFECT OF LIMING ON THE ROOT DEVELOPMENT AND NUMBER OF NODULES



PLATE 3

EFFECT OF INOCULATION ON THE SIZE OF THE SOYBEAN PLANT AND ITS ROOT DEVELOPMENT



PLATE 4

EFFECT OF  $\text{NaNO}_3$  ON THE SIZE AND NODULE FORMATION IN SOYBEANS





PLATE 5

COLONIES OF *B. radicola* 10 DAYS OLD ON ASHBY'S MEDIUM SHOWING THE DIFFERENCES  
IN SIZE OF THE COLONIES PRODUCED BY THE SOYBEAN (UPPER)  
AND THE ALFALFA (LOWER) BACTERIA





## ARE UNUSUAL PRECAUTIONS NECESSARY IN TAKING SOIL SAMPLES FOR ORDINARY BACTERIOLOGICAL TESTS?

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The method of obtaining soil samples for bacteriological study has formed the subject of many investigations since the time of the introduction by Robert Koch of the gelatin plate method into bacteriological technique. In all such studies, the underlying idea has been to guard against the contamination of the samples by dust or by soil from adjoining layers, each of which was to be examined separately. It seems, however, that such contamination was assumed to be appreciable without actual knowledge regarding its extent and its significance. So far as we are aware, no experiments have been conducted to determine the real status of the factor of contamination in soil sampling. In connection with other studies on the "soil columns" of California which were carried out jointly by Hilgard, Loughridge, and the senior author of this paper, the assumption above mentioned was also made when the bacteriological studies were planned and executed. The unusual precautions taken in sampling at that time were emphasized a number of years ago in a paper by C. B. Lipman (1). In planning recently a continuation of the aforementioned bacteriological work, however, it occurred to the senior author that much time, labor and expense might be saved in connection with the necessary soil sampling if the extreme precautions taken in the earlier work could be obviated, by reason of the knowledge that the contamination due to sampling with an auger was an insignificant factor in the work. The idea that the contamination resulting from auger sampling was probably slight seemed to be supported by the logical reasoning that the magnitude and biochemical efficacy of soil flora in a given sample could not readily be influenced by the addition of relatively small numbers of organisms from adjoining soils.

In view of these considerations, further studies of the flora of additional soil columns, to determine the depths below the surface to which bacteria penetrate and are active, were suspended until a proper comparison of sampling with the auger and sampling with unusual precautions from a vertical wall could be carried out. Two soils were chosen for the experiment, one an alluvial loam at Hayward, the other a blow sand at Oakley. The tests used as criteria in this experiment were the following: bacterial counts on bouillon agar, ammonifying power with 0.1 per cent peptone, nitrifying power with soil nitrogen alone, and with soil nitrogen plus sulfate of ammonia (0.2 per cent), and nitrogen fixation in solutions with 2 per cent mannite and in soil with 1 per cent mannite.

The sampling was done as follows: A hole was dug to a depth of 5 feet, and having one vertical wall. The wall was sterilized by a thorough flaming with a plumber's torch. Examination showed that the soil was dried and charred to a depth of  $\frac{1}{2}$  inch. Then, starting at the fifth foot, the flamed surface was scraped away to a depth of 1 inch by a downward cut of a sterile spatula thus laying bare fresh soil. This was then sampled by the resterilized spatula for the total length of the foot. The soil was put directly into previously sterilized cotton-stoppered, glass Mason jars. Each foot was sampled by the same process successively from the fifth foot to the first, the spatula, of course, being resterilized for every foot sampled. The reason for starting with the fifth foot and progressing upward is, of course, obvious, the lowest depth being the region of least bacterial activity. Then, also, soil dislodged and falling down during sampling would not contaminate surfaces to be sampled. The auger samples to be compared with these were taken by boring, with slight precautions, into the soil adjacent to the wall (about 3 inches from it) from which the first set of samples just described were obtained. The auger used was of one of the post-hole type manufactured by Iwan Brothers at South Bend, Indiana. The samples were placed as rapidly as taken into sterile glass fruit jars, cotton-stoppered, and sent to the laboratory for study. The results of the experiment are given in the subjoined tables. Samples 1 to 5, inclusive, are those taken by the sterile spatula from the vertical wall and are marked "aseptic." Samples 6 to 10 are those taken by the auger and are so marked in the tables. For convenience, we shall discuss briefly each set of tests by itself.

#### THE BACTERIAL COUNTS

Considering the appreciable error which inheres in methods of making bacterial counts, it is surprising to find how well the data for both methods of taking the samples in the cases of both soils agree (table 1). The variations which do occur are sometimes in one direction and sometimes in another and in general are too small to be significant. Incidentally, the counts gave a good picture of the numbers of the bacterial population at the different depths in the two soils, and of the relative paucity in bacterial numbers which characterizes the poorer as against the richer soil.

#### AMMONIFICATION DATA

The ammonification results show tendencies similar to those of the bacterial counts (table 2). Again the first foot shows itself to be markedly superior to the lower depths in the Hayward soil, but not so in the Oakley soil. Below the first foot in the Hayward soil and at all depths in the Oakley soil, the ammonifying power seems to be about uniform down to the sixth foot. This is all incidental, however, to the main question at issue here, which seems to be answered unequivocally. The results of the auger and those of the "aseptic" method run practically parallel and more distinctly so than those of the bacterial counts among themselves.

## NITRIFICATION DATA

Fully as striking as the ammonification data, if not more so, are the nitrification results (table 3). For all practical purposes, the two methods of sampling appear to yield identical figures on the nitrifying power of the soil for its own

TABLE 1  
*Bacterial counts*  
Bouillon Agar—incubation one week at 28°C.

METHOD	NUMBER	KIND OF SOIL	
		Hayward	Oakley
		<i>Organisms per gram</i>	<i>Organisms per gram</i>
Aseptic.....	1	100,000,000	2,760,000
	2	65,000,000	1,750,000
	3	5,000,000	1,300,000
	4	5,200,200	1,700,000
	5	4,000,000	860,000
Auger.....	6	130,000,000	2,500,000
	7	30,000,000	1,900,000
	8	4,400,000	1,450,000
	9	5,400,000	1,400,000
	10	3,000,000	900,000

TABLE 2  
*Ammonification*  
0.1 per cent Peptone—50 grams soil—incubation one week at 28°C.

METHOD	NUMBER	AMMONIA NITROGEN PRODUCED	
		Hayward soil	Oakley soil
		<i>mgm.</i>	<i>mgm.</i>
Aseptic.....	1	17.78	8.26
	2	8.12	9.52
	3	7.28	9.66
	4	5.30	9.66
	5	5.30	8.82
Auger.....	6	18.20	8.40
	7	7.70	9.38
	8	6.86	9.24
	9	6.02	8.68
	10	5.32	8.82

nitrogen as well as for that of sulfate of ammonia. This is true for both soils. Incidentally, again, it is interesting to note the superiority of the first foot in this case of both soils as against the lower depths. The nitrifying powers of the

Hayward soil stand out in sharp contrast, however, with the ammonifying powers of the same soil as above determined. The successive depths decrease rapidly in their power to produce nitrate, but they do not do so as regards ammonia production, except as between the first foot and all the others which appear to be about uniform. In the Oakley soil, on the other hand, the nitrification data are very similar in general trend to the ammonification data, except that in the case of the sulfate of ammonia nitrogen, the first foot of soil, behaves, in general, like that of the Hayward soil.

#### NITROGEN-FIXATION DATA

The only significant data, in one sense, which have been adduced from the nitrogen-fixation studies are contained in the results for the Hayward soils (table 4). These seem to indicate, both in the solution and in the soil cultures, that there is no appreciable contamination of one layer of soil by another when the samples are taken by means of the auger. In the latter respect, however, even the data for the Oakley soil, while showing little or no fixation of nitrogen, are significant, since the samples taken by the auger yielded virtually the same results as those taken "aseptically." On the other hand, it is obvious that not much contamination of one layer of soil by another with nitrogen-fixing bacteria could be induced through the auger method of sampling when no nitrogen-fixing bacteria were present. There having been no *Azotobacter* membranes observed in the mannite solution, cultures with the Oakley soil, except as noted in two cultures which probably became contaminated, it is not surprising that little or no nitrogen fixation was obtained. Absolutely none was obtained in the soil cultures and less than half a milligram in every case in the solution cultures. This discrepancy and other circumstances discussed render it probable that the data for the soil cultures reflect more correctly the status of the Oakley soil with respect to nitrogen fixation.

In the Hayward soil, *Azotobacter* membranes were found in all of the solution cultures, and were about of equal vigor for the cultures prepared from the auger and the "aseptically" collected samples. In the solution cultures, nitrogen fixation seemed to be most vigorous in the first 3 feet and less so in the last 2 feet. In the soil cultures, however, nitrogen fixation was very marked in the first 2 feet and fell off sharply in the other 3 feet in which it appeared to be uniform. There was general agreement in this respect between the cultures made from the two sets of samples tested.

#### SUMMARY AND CONCLUSIONS

It seems clear from the foregoing tables and discussions that there is no necessity for taking unusual precautions in sampling soils for bacteriological work. This is at least true for all such ordinary tests as are made in most laboratories to obtain certain bacterial coefficients of soils. The ordinary removal of samples from the field soil by augers, with slight precaution, seems to intro-

duce no contamination from one depth of soil to another, as the auger passes downward. Tests for bacterial counts, ammonification, nitrification, and nitrogen fixation on soil columns 5 feet in depth give strong evidence in support of the foregoing assertion. The reason for the evident failure of the auger method of sampling to affect the soil from layer to layer by direct contamination is probably to be found in the inadequacy of the relatively small numbers of organisms introduced, to the task of affecting to a perceptible degree the much larger and more firmly established flora already existing in a given soil stratum. The investigators, including the senior author, who have attempted to devise methods for preventing contamination of soil samples by taking unusual pre-

TABLE 3  
*Nitrification*

METHOD	NUMBER	NITROGEN AS NITRATE PRODUCED FROM TWO SOURCES OF NITROGEN					
		Hayward soil			Oakley soil		
		Nitrate in soil initially	Nitrate produced from soil nitrogen	Nitrate produced from ammonium sulfate	Nitrate in soil initially	Nitrate produced from soil nitrogen	Nitrate produced from ammonium sulfate
		<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Aseptic.....	1	0.35	1.50	10.00	Trace	0.25	1.00
	2	Trace	1.00	2.75	Trace	0.20	0.25
	3		0.35	1.50	Trace	0.25	0.25
	4		0.25	0.75	Trace	0.25	0.25
	5		0.35	0.75	Trace	0.40	0.35
Auger.....	6	0.35	1.50	10.00	Trace	0.25	0.80
	7		1.00	2.50	Trace	0.30	0.45
	8		0.35	1.50	Trace	0.15	0.30
	9		0.25	0.75	Trace	0.25	0.15
	10		0.35	0.80	Trace	0.30	0.25

cautions, have based their efforts on what seems now to be an erroneous assumption. The latter was in turn due to a lack of knowledge as to whether or not contamination in ordinary soil bacteriological work is a factor of significance. From the experiments which we have carried out in a study of this question, the following conclusions, among others, have resulted:

1. For ordinary bacteriological work on soils, no special precautions are necessary in taking soil samples.

2. Samples taken to a depth of 5 feet each with an ordinary soil auger, and by the "aseptic" method from a flamed vertical wall of a pit, respectively, show no significant dissimilarity in bacterial counts, ammonifying power for peptone (0.1 per cent in soil), nitrifying power, and nitrogen-fixing power. That the sampling itself from the vertical wall constituted a "precautionary" method is indicated by the striking contrasts which characterize the comparisons made above in various respects of the different layers of a given soil.



3. All attempts at devising methods of soil sampling for ordinary soil bacteriological work have been based evidently upon the erroneous assumption that dangers from contamination in such work are considerable.

4. The soil flora in a given sample of soil seem to be so large, so characteristic, and so firmly established and adapted to the conditions under which they are found that the introduction of relatively small numbers of contaminating organisms into that sample are without perceptible effect on the original flora as shown in ordinary tests on soils.

5. Incidentally to the main conclusions of this paper, it is also to be noted that in the two soils studied, as in others described in a paper above cited, large

TABLE 4  
*Nitrogen fixation*

METHOD	NUMBER	NITROGEN FIXATION IN SOLUTION 50 CC. MANNITE SOLUTION—INCUBATION 2 WEEKS AT 28°C		NITROGEN FIXATION IN SOIL 100 GM. SOIL + 1 PER CENT MANNITE—IN- CUBATION 3 WEEKS AT 28°C	
		NITROGEN FIXED PER GRAM MANNITE		NITROGEN FIXED PER GRAM MANNITE	
		Hayward soil	Oakley soil	Hayward soil	Oakley soil
		<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Aseptic.....	1	10.99	0.57	14.00	0.00
	2	9.31	0.49	19.60	0.00
	3	8.47	0.49	5.60	0.00
	4	4.20	0.57	4.20	0.00
	5	5.32	1.54*	7.00	0.00
Auger.....	6	10.08	0.57	16.80	0.00
	7	7.49	0.57	21.00	0.00
	8	7.35	0.77	5.60	0.00
	9	6.51	1.12*	7.00	0.00
	10	5.32	0.84	8.40	0.00

\*Probably contaminated. *Azotobacter* film.

bacterial numbers and notable bacterial activity are to be found at relatively great depths in soils of the arid region. This probably is very different from the conditions which characterize soils of the humid region.

6. Despite the evidence mentioned in the foregoing conclusion, respecting the great depths to which bacteria penetrate in arid soils, it is to be noted that in practically all tests, the surface foot of soil is by far the most active bacterio-chemically speaking, and is by far the most densely populated. In some phases of the soil's bacterial activity, however, the second foot approaches or equals the first foot. As a rule, the soil layers from 2 feet down to 6 feet are nearly uniform in bacterial population and activity in a given soil.

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# THE IMPORTANCE OF MOLD ACTION IN THE SOIL<sup>1</sup>

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## INTRODUCTION

When a group of microorganisms is studied in relation to soil fertility, the first question that presents itself is: What is the nitrogen metabolism of these organisms? What part do they play in the nitrogen cycle of the soil? The largest number of investigations dealing with soil microorganisms, whether in pure or in mixed cultures, whether studied in solution or in the soil, in the laboratory or in the field, are concerned with the possible nitrogen changes in the soil produced as a result of the activities of these organisms. Since the nitrogen changes in the soil are among the most important ones in the study of soil fertility, the wildest speculations have often been made in an attempt to interpret the nitrogen changes produced in the laboratory upon artificial culture media from a few organic or inorganic substances by microorganisms and account thus for the different soil processes which are important from the point of view of soil fertility problems. Fewer investigations were devoted to the study of the carbon, phosphorus, iron, sulfur, and potash changes in the soil as a result of microbial activity.

The very methods employed in soil bacteriological investigation can be subjected to a great deal of criticism. Without going at present into a detailed discussion of the different methods used in the study of nitrogen metabolism of the soil flora as a whole, an attempt will be made in this paper to take up the study of the metabolism of molds which commonly occur in the soil. This may help to throw some light upon the part played by these organisms in different soil biological processes.

From the early period of soil microbial investigations up to the last four or five years, nearly all the attention of the soil bacteriologists was centered upon the study of bacteria, entirely neglecting the other groups of microorganisms, although these must have come to their attention here and there. The fact could not be overlooked that the soil has an abundance of molds, actinomycetes, protozoa, rotifers and, under certain conditions, algae, which exist in the soil in an active state. Whether the cause for neglect was the use of media more favorable to bacteria than to the other microorganisms for their isolation from the soil; whether it was due to the fact that the bacteria form the

<sup>1</sup> The species of fungi isolated from the soil belong to widely scattered groups; the common term "mold" is applied here collectively to these organisms, although no sharp limitation can be placed on the use of the term.

more numerous group of soil organisms, and, as always happens in such cases, the attention is centered upon the more frequently-occurring types; whether the important advance along bacteriological lines from the point of view of pathogenicity to men and animals has centered upon it so much of the attention even of the soil bacteriologist, that the other organisms (non-bacterial) were neglected—whichever was the cause, it was only within the last half-dozen years that the great abundance of other microorganisms in the soil besides bacteria, has been demonstrated and an attempt has been made to explain their probable part in soil fertility.

The work of Russell and his associates (48) upon the possible part played by protozoa in soil fertility has called forth a series of investigations on the occurrence of soil protozoa and their activities. The algae of the soil were studied by several investigators. The occurrence of actinomycetes and their possible rôle in the soil have been recently summarized in several papers. It will be the object of this paper to point out several metabolic processes of the molds occurring in the soil and thus attempt a suggestion as to their part in soil fertility. An extensive bibliography on this subject can be found elsewhere (56). The several soil biological processes will be taken up and, by comparing the activities of the molds with those of bacteria, the important transformations which the organic matter and the mineral matter undergo in the soil and which may be due to the action of the molds, will be discussed.

The observations and conclusions are based on the work of different investigators, but chiefly on that of the writer.

#### OCURRENCE OF MOLDS IN THE SOIL

It has been definitely established (56, 58) that the molds are common inhabitants of the soil and form a large and important group of the soil flora.

Hundreds of species of molds have been isolated repeatedly from the soil; it has been found that many molds occur in different soils, under different topographic, climatic and crop conditions. The same species have been isolated by a number of investigators in different European countries and from numerous soils in this country. A number of new species never encountered before were isolated from the soil, which would serve as additional proof that some of these are typical soil organisms. Although as many as 1,000,000 fungus colonies developed from 1 gm. of soil in some cases, this cannot be taken as proof that so many pieces of mycelium were present in that quantity of soil, but merely that a mass of spores were present and each of these spores developed into a colony when the soil was shaken with water and plated out. The method of determining the number of molds in the soil is very inefficient, and since usually only two or three plates are made from each dilution, the probable error is much greater than anything that should be allowed for exact work. This was the reason why the writer, in one of the earlier publications (58), attached little importance to the plate count of molds in the

soil. And before more exact work has been done along this line and a large enough number of samples and soils have been studied, we shall not be able to state definitely as to how many molds (or rather mold spores and pieces of mycelium) can be expected in each gram of soil; even then, the uncertainty will exist because of the fact that at one spot an organism might have sporulated, which would give a tremendously large number of spores, not indicating, however, any predominant rôle for this organism over others which may occur in much smaller numbers. For example, such organisms as the *Aspergilli* or *Penicillia*, which produce a very large number of spores separating with relative ease, are often found in predominant numbers, while at a different period of the year these organisms may be entirely absent.

This may account for the statement of Hagem (20) that certain *Aspergilli* occur in larger numbers in the soil than all the *Mucors* taken together. This statement was made for Norway soils, and as the writer pointed out elsewhere (58), the *Aspergilli* are more abundant in a warmer climate, while the colder climates show a greater proportion of *Mucorales* in the soil.

A detailed discussion as to the occurrence of the different genera and species of fungi in different soils, as well as the methods of isolation, both in vegetative mycelial and spore forms can be found elsewhere (56, 58). In reviewing the work of others and his own, the writer concluded that there is a fungus flora of the soil and that many molds occurring in one soil will also be found in other soils, under different conditions of cultivation topography, origin and climate. The fact was pointed out that the Northern soils have a greater abundance of *Mucorales* and *Penicillia*, while the Southern soils are richer in *Aspergilli*; *Trichodermae* are found in large numbers in acid soils; *Fusaria*, *Cladosporia*, *Chaetomia*, *Alternaria*, etc. will be always found in most soils, if a large enough number of samples are studied and care taken in isolation. The list will no doubt be extended a great deal with a more intimate knowledge of the different more or less obscure forms of fungi present in the soil which require the careful study of the specialist in the different groups of these organisms.

Ramann (44) found that molds develop readily in acid soils and are more active in the forest and in the compact poor soils, while bacteria predominate in loose soils, rich in nutrients, cultivated and fertilized. Hagem (20) stated that in the well cultivated lands containing little humus the bacteria play an important part and occur in predominant numbers and the molds are of minor importance; while the upper layers of pine forests, rich in humus, contain a large number of molds. The rainy seasons of the year, fall especially, favor the surface growth of these organisms, otherwise they live and sporulate below the surface, among the plant residues and living roots. Since the last publication of the writer (58) there appeared a paper by Pratt, who isolated from Idaho soils a number of organisms, particularly *Mucors*, *Fusaria* and *Penicillia*, many of which were isolated by the writer previously from different soils of North America.

The fact that molds are found in the soil would not warrant as yet any extensive study of these organisms, before it has actually been demonstrated that they are not only present in the soil, but actually live there and produce mycelium, which would necessitate their taking an active part in the different biological transformations taking place in the soil. The writer (57) suggested a method by which it could be demonstrated that molds actually live in the soil, and, although not all the organisms found in the soil could be demonstrated by this method to have produced mycelium (curiously enough, the organisms found in the largest numbers, such as *Aspergilli* and *Penicillia*, could be demonstrated in the soil by this method only in very few cases), a number of groups were found to be present in the soil in an active state.

Conn (9), using a direct microscopic method for examining soils, found that mold mycelium is present in the soil only to a very limited extent. If plate counts of molds present in the soil do not have the same significance as plate counts of bacteria, as pointed out by Conn (9) who was preceded in this by the writer (58, p. 571), the microscopic method of Conn (9) is probably of not much greater value in this respect. The relative numbers of spores and hyphae of typical soil molds is such that, as Conn himself states, 3000 microscopic fields would have to be examined before a piece of mycelium could be found, while enough spores were produced by the same organisms to give a plate count of 300,000 per gram of the soil in which it would be grown.

Brown (4) repeated the work of both the writer and Conn and demonstrated that mycelium was present in all soils examined. Conn, using very small quantities of soils, namely 10 mgm., could easily have overlooked the mycelium that might have been present, although Brown stated that by using the writer's method he could demonstrate mold mycelium not only in 10 mgm. but also in smaller quantities of soil. Thom and Church (53) observed that different *Aspergilli* and *Penicillia* planted upon the surface of sterilized soil are capable of growing into the soil to considerable depths and even of producing spores.

The fact should not be overlooked that most of the investigations upon soil molds were made by botanists, who were either interested more in the types of the organisms as such, or as to their possible pathogenicity to plants, but not from the point of view of soil fertility. To be able to interpret the proper part played by these organisms in the soil we must study them not only as vegetative or other forms of plants, but as living organisms which exist in the soil, and by their metabolic processes help in the various transformations through which both organic and inorganic soil constituents undergo and thus affect the fertility of the soil.

The universal presence of molds in the air, on decaying fruit and different forms of organic matter, has brought them early to the attention of the biochemist, who used some of these organisms in investigations on metabolism. But since the fact has been established that these organisms exist in the soil, their biochemical activities could then be interpreted so as to understand their possible part in the fertility of the soil.

## NITROGEN-FIXATION

The question of nitrogen-fixation by molds commonly isolated from the soil was under dispute, some investigators demonstrating that it was positive, while others could not find any fixation at all. The very recent work of Goddard (19), Waksman (56), Duggar and Davis (17) and Chambers (7), using more exact chemical methods and taking all precautions to eliminate any possible error that may creep into such experiments, has shown that the fungi commonly occurring in the soil, such as the different species of *Aspergilli*, *Penicillia*, *Macrosporia*, *Alternaria*, *Mucors* and others do not fix any atmospheric nitrogen. The amount of nitrogen-fixation by molds claimed to be positive by certain recent workers is so slight (below 5 mgm. per 50 cc. of solution) as to be of practically no importance in soil fertility when compared with the nitrogen-fixing bacteria. The slight quantities of nitrogen-fixation obtained by several workers may possibly be explained by the fact that no precautions were taken to eliminate any nitrogen compounds from the air of the laboratory, to have the chemicals carefully analyzed and to make a large enough series of determinations so as to eliminate any possible error. A detailed discussion of the literature on the subject can be found in the papers of the writers mentioned above. The mycorrhiza fungi, as shown recently by Peklo (39) and certain other fungi, not commonly occurring in the soil, such as cultures of *Phoma Betae*, studied by Duggar and Davis (17), may show a definite nitrogen-fixation.

It can therefore be definitely concluded that, with the exception of certain organisms, which are not very common in the soil, the typical soil molds do not play any direct part in the economy of the nitrogen enrichment of the soils.

## NITRIFICATION

Neither nitrite nor nitrate formation has ever been demonstrated for any of the molds, so that this important process in soil fertility will have to be eliminated, as well as the nitrogen-fixation, from the field of mold activities in the soil. As will be demonstrated later, the nitrogen activity of the molds tends rather to a process of reduction than to oxidation. The breaking down of the complex proteins to proteoses and peptones, then to polypeptides and amino acids, and finally to ammonia, is accomplished by means of molds just as rapidly and thoroughly as by means of protein-decomposing bacteria. Whether this is accomplished as a result of the feeding of the organism, or by means of enzymes, and also whether it is due to the action of the organism upon the nitrogen part of the protein molecule or its carbon part, will be taken up later. All that can be pointed out here is that the organism decomposes the proteins of the soil with the liberation of ammonia, which is either left in the soil or absorbed by the organism in the process of the building up of microbial proteins. There is no need for the mold to oxidize the ammonia

into nitrates, since the ammonia as such is just as good a form of nitrogen for molds as nitrates are, when other conditions are equal.

Schloesing and Müntz (51) showed in 1878 that *Aspergillus niger* and other molds do not nitrify. The molds may exert an indirect effect upon nitrification, due to the fact that in acid soils the normal bacterial activities may be repressed and the growth of molds encouraged, as pointed out by Hall and his associates (21).

#### AMMONIFICATION

When we approach the subject of the disintegration of organic matter in the soil, particularly the first stages of decay, the molds are found to play a very important part: both the disintegration of nitrogenous organic compounds, which will be considered under the above heading and the decomposition of celluloses, hemicelluloses and starches in the soil taken up later.

The subject of ammonification has been often misunderstood and such importance attached to the information obtained from the determination of ammonia in the soil that could not possibly be warranted at all by the data. It has not been even definitely settled as yet as to what part ammonia plays in the metabolism of microorganisms. Czapek (12), in an exhaustive study of the utilization of different substances as sources of nitrogen by molds, found that amino acids are used much more economically than other nitrogen compounds. He assumed that molds, in building up their proteins from simple nitrogen compounds such as ammonia salts, have to produce first amino acids, and these are used as building-stones for the production of complex proteins; if amino acids are offered as sources of nitrogen, the organism will be spared the waste of energy which would be necessary if it had to build up its proteins from simpler nitrogenous substances. Hagem (20) claimed that the utilization of amino acids by molds takes place in the following manner. By the addition of water to the amino acid, ammonia and the corresponding oxy-acid are produced; and the protein synthesis takes place from the ammonia thus formed.

Whatever may be the process of formation of complex proteins by molds one thing is certain—that ammonia is left in the medium as a waste product of the protein metabolism of the organism. But even assuming that, we cannot state definitely that ammonia produced by a certain organism or a mixture of organisms will point to a definite condition and interpretation, because the ammonia accumulated as a result of the action of one organism from one nitrogen compound will depend not only on the source of nitrogen, but also on the amount and availability of carbon compounds present in the medium. When we therefore claim that a certain form of life or a complex mixture of different forms, such as a soil flora, will produce so much ammonia from peptone, dried blood, cottonseed meal or other organic nitrogen compounds, and try to interpret from it the activities of the given flora, we may be entirely wrong even if the ammonia produced could be taken as a factor

of the metabolism of the organisms, because the amount of ammonia produced may depend more on the character of the carbon compounds present than on the source of nitrogen. Hagem (20) has shown that although certain amino acids can be used both as sources of energy and nitrogen by molds, the ammonia produced will depend on the presence of carbohydrates in the medium. Kendall and his associates (26) have studied in detail the protective action exerted by available carbohydrates upon the decomposition of proteins by microorganisms. The writer (59, 60), in taking up the same work and applying it to the study of molds isolated from the soil, has tried to develop the theory further and apply it to the changes of the organic matter in the soil due to the action of microorganisms. The bacteria and molds will attack the protein molecule in the soil to derive from it the nitrogen needed for structural purposes, if available carbohydrates are present to supply the energy required; only small quantities of ammonia will be liberated under these conditions. But, in the absence of available carbohydrates, the organisms will attack the protein molecule not only as a source of nitrogen, but also as a source of energy; since the energy requirement of the organism is greater than the nitrogen need, only a small part of the nitrogen of the protein molecule will be used for the building up of the proteins of the microorganism, and the greater part of the nitrogen will be left in the medium as a waste product in the form of ammonia. We should therefore take up the study of ammonification by molds with this idea in mind: the ammonia merely indicates the activities of the organism in the presence of a certain source of carbon and nitrogen. It will indicate, as Hagem (20) stated, the mineralization of the organic matter.

The early workers on ammonification studies used both molds and bacteria. Müntz and Coudon (36) and Marchal (32) demonstrated in 1893 the fact that soil molds are as active ammonifying organisms as bacteria. Marchal (32) even attempted to ascribe the ammonia production in soils (particularly acid soils) chiefly to the action of molds. But following the work of these investigators, the greatest attention has been paid to soil bacteria. Only here and there a paper appeared calling attention to the occurrence of molds in the soils or to the chemical changes produced by them when grown on artificial culture media. Butkewitch (6) observed that in the decomposition of proteins by molds, ammonia and amino acids were formed, among the latter tyrosin and leucin being identified. He has been able to show that, by changing the cultural conditions of the same organism, either a rapid transformation of the peptone into ammonia with the production of only a small quantity of amino acids, or a slow accumulation of ammonia with a large quantity of amino acids takes place. The accumulation of ammonia runs parallel with that of oxalic acid; when the latter is neutralized by means of  $\text{CaCO}_3$ , the ammonia accumulation is suppressed and other nitrogen compounds such as tyrosin and leucin, appear in the substratum. He agrees with Czapek, that amino acids are used directly by the organisms, while ammonia is a by-product



in the decomposition of the complex nitrogen compounds. The least energy was spent by the organism in utilizing amino acids, more energy was used when ammonium salts and nitrates were offered as a source of nitrogen, and still more when peptone and egg-albumen were the nitrogen sources.

Iwanow (23) has shown that, in the decomposition of the seeds of yellow lupins by *A. niger*, small quantities of tyrosin, larger amounts of leucin and still more ammonia, in the form of ammonium oxalate, were produced. Kosyachenko (31) found among the products of decomposition of the proteins of peas by the same organism tyrosin; leucin; the hexone bases histidine, arginine, and lysin; and ammonia. Hagem (20) has shown that, in the utilization of amino acids and peptone by *Mucors*, ammonia is always produced. Kappen (24) found that several molds decompose cyanamide with the production of ammonia. Other references to the work on the decomposition of organic matter by molds and liberation of ammonia will be found elsewhere (56). McLean and Wilson (34) found that soil molds produce in pure culture a much greater accumulation of ammonia than bacteria. Waksman and Cook (62) confirmed these observations and called attention to the fact that the accumulation of ammonia as a result of mold activities seems to take place in definite cycles and expressed the idea that there may be a relationship between the growth period of the organism and ammonia accumulation. Further information on the subject of decomposition of organic matter by fungi isolated from the soil can be found in the work of the writer (56), who has shown that the growth of molds on artificial culture media affects the ability to decompose organic matter by some molds and not by others. Kopeloff (28) found that an increase in the number of mold spores inoculated into the soil is responsible for a proportionate increase in ammonia production up to a certain point; additional information on the environmental factors influencing the decomposition of organic matter added to sterilized soil by soil fungi are supplied by Coleman (8). The decomposition of urea, uric and bipuric acids and glycoll by molds with the subsequent liberation of ammonia was studied by Kossovich (30).

Although, as shown above, the production of ammonia from organic matter, particularly when this has been added to sterilized soil, making the conditions distinctly different from normal, cannot be taken as a true indication of the rôle of these organisms in soil fertility, we are still able to obtain some information from these studies. In nearly all cases reported, some molds, such as *Trichoderma Koningi*, *Monilia sitophila*, and others are able to decompose the organic matter much more rapidly than the strongest ammonifying bacteria known, such as *Bacillus mycoides* or *Bacillus subtilis*. Even if the ammonia is looked upon only as an indication of the amount of organic matter decomposed by the organism, some of the molds, commonly occurring in the soil, are found to possess distinctly greater powers of decomposing the organic matter than do the bacteria. The writer (60) has shown that *A. niger* grown in culture media containing peptone or asparagine as a source

of nitrogen will bring about a rapid decomposition of the material, although the total ammonia production will depend entirely on the amount of available carbohydrate present. It was also pointed out that the ammonia production by *A. niger* is an autocatalytic phenomenon. A word should be said here that, although it has been definitely established by Miyaki (35) for bacteria and by the writer (60) for molds that the ammonia production obeys the law of autocatalysis, as shown by a mathematical interpretation of the results, it has not been demonstrated as yet that a catalyst actually exists, or that the action and counteraction combining to give the curve of autocatalysis may not be merely a result of the growth of the organism (action) and the injurious action of some of the by-products in the culture medium (reaction).

The action of the molds upon the nitrogenous organic matter of the soil consists, therefore, in the mineralization of these materials, with the production of ammonia and the building up of fungus proteins. The ammonia is either used by the higher plants as such or oxidized by the nitrifying bacteria into nitrates and used by the plants in this form, or absorbed again by the microorganisms of the soil either in the form of ammonia or even as nitrates and used for the production of the complex microbial proteins, as will be shown later.

#### DECOMPOSITION OF CARBON COMPOUNDS IN THE SOIL

The molds play an active part in the decomposition of celluloses and other carbon compounds in the soil; the importance of this process is well known to the student of soils, since the addition of green and animal manures, plant roots and other residues, necessitates this process before the minerals and nitrogen compounds can be brought to a form, in which they could be either taken up by the higher plants directly or after they have undergone another transformation due to the action of other groups of molds or bacteria. Celluloses, pectins, vegetable gums and other similar substances are rather inert and cannot be attacked by all microorganisms harbored in the soil; among those that are able to do it, the molds occupy a prominent place.

The work of Van Iterson (55), Koning (27), Dascewska (13), McBeth and Scales (33), and others has established the fact that the rôle of molds in the soil in the destruction of cellulose has been greatly underestimated; a number of molds were isolated from soil which decompose cellulose very rapidly. The different molds differ greatly in this respect: while some, such as different *Penicillia*, *Aspergilli*, *Trichodermae*, and others dissolve the cellulose very rapidly, others, such as the *Mucorales* (20) cannot attack it at all. Schellenberg (50) demonstrated that the ability of the molds to dissolve different celluloses does not depend on the solubility of these in acids, but on the chemical composition of the substance. Hagem (20) found that the *Mucorales* can use, out of all the carbon compounds found in the soil, only the pectin bodies and the mono-saccharides, and partly the disaccharides, while the cellu-

loses and hemicelluloses are left intact. He concluded that the Mucorales must take only a limited part in the decomposition of carbon compounds in the soil, when compared with the other molds and bacteria. Out of nearly a hundred organisms tested by the writer for the ability to decompose cellulose according to the method of McBeth and Scales (33), only the Mucorales and a few other organisms (several *Fusaria* and *Sporotricha*) produced little or no decomposition of the cellulose, while the *Trichodermae*, *Cephalosporia*, *Aspergilli*, *Penicillia*, *Verticillia*, and others produced a strong or very strong decomposition. A detailed discussion of the action of different compounds would be here out of place; it may only be stated that nearly all the simple and complex organic carbon compounds in the soil can be attacked by one or another group of soil molds, and these, through this action, play an important part in the fertility of the soil.

The question of the humin substances of the soil may be brought up here. Ramann (44) states that Nageli, Hoppe-Seyler, Kostytschew, Müller and others concluded that the molds are the proper humus builders in the soil. The fallen leaves, at the end of the vegetative period in the fall, are found to be penetrated with mold mycelium, which decomposes the leaves readily, with the production of humic substances. Hoppe-Seyler (22) claimed in 1889 that no plant or animal is able to use humin substances as food, and no bacterium can bring forth their decomposition; they afford an habitat and substratum to many bacteria, molds, algae and animals. Reinitzer (45) and Nikitinsky (38) have shown that humin substances cannot serve for molds and bacteria both as a source of carbon and nitrogen, but in the presence of available sources of carbon, they can be used as a source of nitrogen. The value of the study of humin substances in the soil and the action of microorganisms upon them has to be proven as yet, since they are artificial in nature, and the fact has not been even established as yet that they exist as such in the soil, and are not merely decomposition products due to the action of chemicals upon the soil.

As to the utilization of starch and production of amylases, the molds seem to be very active. *Aspergillus Oryzae*, *Aspergillus niger* and other *Aspergilli* and *Penicillia* have been found to produce very active starch-splitting enzymes.

The production of carbon dioxide can be taken as an index of the decomposition of soil organic matter. It is a much more accurate index of the biological transformations going on in the soil, since once it is liberated, it is not utilized again by the soil microorganisms, while ammonia and nitrates which are used more commonly by the soil bacteriologists, as indicating soil biological processes, can be utilized again by other organisms in the soil. Neller (38) made recently some interesting observations on the correlation between the production of carbon dioxide and accumulation of ammonia by soil organisms. The molds tested oxidized more of the carbon and produced less ammonia than the bacteria did; mixed soil infusions resembled the molds in the low accumulation of ammonia, but produced larger quantities of carbon

dioxide. Neller, therefore, suggested that the soil molds were the more active components of the natural soil flora, and a low accumulation of ammonia with alfalfa as the source of carbon, may not necessarily indicate a low activity, contrary to the usual conclusions of soil bacteriologists, who took only ammonification, nitrification, or bacterial numbers, as an indication of soil biological processes. A very active production of carbon dioxide from organic matter by molds isolated from the soil was made recently also by Potter and Snyder (40), who have demonstrated that several fungi isolated from the soil liberated nearly as much carbon dioxide from sterilized soil as did a mixed soil flora (soil infusion), both in the presence and absence of dextrose.

These two observations together with the work of the writer (59, 60) on the action of available carbohydrates upon the ammonia production by molds will help to throw a great deal of light upon the activities of these organisms in the soil. The molds attack the carbohydrates very readily, perhaps even more readily, concluding from these few observations, than the bacteria do. This rapid decomposition of the carbohydrates, both complex and simple, indicates a strong activity and rapid need and utilization of energy; the index of the rapid respiration is the carbon dioxide production. The nitrogenous organic compounds may be decomposed only to a smaller extent than they would be in the absence of these available carbohydrates; very little ammonia is therefore produced. But even when the nitrogenous compounds are decomposed rapidly and when a large amount of ammonia would be expected as a waste product of protein metabolism of the organisms, the ammonia will not be accumulated in the medium, but will be used, in its turn, by the organism for the further building up of mold protein, as long as carbohydrates are available to supply the energy. The ammonia will therefore not accumulate in the soil from the nitrogenous compounds, in the presence of available carbohydrates, for two reasons: first, less of the nitrogenous substances will be decomposed and, therefore, less ammonia will be left as a waste product, as shown by the writer (59, 60); second, the ammonia will be further utilized by the organism in the building up of the fungus protein, because of its rapid growth, as shown by the carbon-dioxide production; this can be readily seen from the data obtained by Neller (37). Of course, more information is necessary before we may be able to construct an exact theory as to chemical changes taking place in the soil and underlying soil fertility. One thing is certain that *we will have to construct our theories on soil fertility, particularly on the nitrogen part of it, not only from the point of view of nitrogenous manures and fertilizers and nitrogen content of the soil, but also by taking into consideration the nature and amount of carbon compounds added to the soil.* A study of the action of pure and mixed cultures of molds, as well as of bacteria, will help us to throw light on this subject. These observations clearly indicate *of how little value is the study of ammonia production by different pure or mixed cultures of soil organisms, when other factors are not taken into consideration.*

## UTILIZATION OF NITROGEN COMPOUNDS

A knowledge of the utilization of nitrogen compounds by molds in the soil is important for a thorough understanding of soil biological processes, particularly from the point of view of soil fertility problems. Besides a knowledge of the production of ammonia, nitrates, and other simple nitrogenous compounds in the soil, or the addition of these in the form of an artificial fertilizer, we have to keep in mind that the lower organisms present in the soil will always compete with the higher plants in utilizing these compounds and converting them into complex proteins. The utilization of the different forms of nitrogen compounds has attracted the attention of many chemists, and some very interesting work has been done along these lines. Two molds chiefly have been studied: *Aspergillus niger*, whose identity can be recognized without much difficulty, although it has been fairly well established that different strains of this organism exist, which differ somewhat in their biochemical activities (54); and *Penicillium glaucum*, which is a rather vague term, since there exist many species of green *Penicillia* which could be termed *P. glaucum*. Czapek (12) made an extensive study of the nitrogen compounds that can be utilized by *A. niger* for the building up of the fungus protein; albumoses and peptones are utilized; peptones are produced out of amino acids, and these are in turn condensed to proteins. Since amino acids are necessary for the synthesis of fungus proteins, therefore, he argued, amino acids are the best sources of nitrogen for these organisms, since they will be spared the expense of energy which would be necessary to produce the amino acids out of simpler nitrogenous compounds. The nutritive value of the other nitrogen compounds depends on how easily they can be transformed into amino acids. Puriewitsch (42) started out with the idea that the more easily a substance is utilized by an organism, the fewer will be the stages through which the substance will have to undergo and the less will be the expenditure of energy. As a measure of this expenditure of energy he used the carbon dioxide production per unit of dried body weight of the organism. He confirmed the observation of Czapek (12): the utilization of energy was least for amino acids; ammonium derivatives followed, then nitrates, and peptone, and finally egg-albumen requiring the largest expenditure of energy. Raciborski (43), Hagem (20), Abderhalden (1) were of the opinion that the amino acids (also nitrates and nitrites) are first reduced to ammonium salts and in this form utilized for the production of proteins. Raciborski (43), Puriewitsch (41) and Brenner (3) stated that nitrites are poisonous for *A. niger* in an acid solution, while in an alkaline medium assimilation is positive and in some cases even just as good as nitrates. Ritter (46) found that the ability of an organism to assimilate nitrogen from inorganic ammonium salts is in direct relation to the ability of the organism to withstand the mineral acid liberated.

Without going into a detailed discussion on the assimilation of ammonium salts, nitrites, nitrates, and other inorganic and organic nitrogenous compounds by molds [such a discussion can be found in the paper of Brenner (3),] we may merely note here that this is of great importance from the point of view of soil fertility problems, since these organisms will use up the nitrogen compounds in the soil available for higher plants and will exert thus a very unfavorable action. This was pointed out by several investigators, particularly by Rothe (47), who stated that the molds exceed the bacteria and actinomycetes in acid as well as in neutral media in the assimilation of the available nitrogen and storing it away in an organic microbial form; under favorable circumstances, namely in the presence of calcium carbonate, large quantities of nitrogen added to the soil in the form of ammonium salts are transformed by these organisms into very insoluble nitrogen compounds. Hall and associates (21) stated that, under certain conditions, molds must compete with higher plants for the nitrogen added to the soil.

Sullivan (52) suggested that the complex organic substances found in the soil may be a result of the growth of the mold mycelium. The nitrogen which would have been otherwise available for higher plants may be transformed into an insoluble, concentrated, and mostly undecomposable form. This question is discussed in detail by Ehrenberg (18). He stated that fungus protein is much less available for further decomposition than bacterial protein; the spores contain a large quantity of nitrogen stored away in an unavailable form. The disappearance of the available nitrogen added to the soil in the form of ammonium salts or nitrates is to be looked for more in the mold metabolism rather than bacterial. Denitrification due to the action of molds is discussed in detail by Ehrenberg (18). When ammonium salts are added to the soil together with manure, the large quantities of energy material introduced allow a rapid growth of the soil molds, which assimilate a large quantity of ammonium sulfate nitrogen, thus preventing it from becoming available to higher plants.

We can thus see that the molds of the soil may produce a very unfavorable effect upon soil fertility in competing with the higher plants for available nitrogen compounds, particularly in the presence of large quantities of available carbohydrates. Although this injurious action, under certain conditions, cannot be denied, even if the extent of it has not yet been definitely established, two other factors should be considered here which may counterbalance the possible injury to higher plants. First, we know very well that an excess of ammonium salts and nitrates in the soil will lead to large losses due to natural or artificial irrigation and drainage, particularly in humid climates; the utilization of some of these nitrogen salts by the soil molds, bacteria and other organisms may serve for the conservation of a great deal of this nitrogen in the soil. Second, the autolysis of mold mycelium, resulting in the splitting off of the fungus protein with the liberation of ammonia, as shown by Dox and Maynard (16), Brenner (3) and the writer (60) will tend toward the

giving back to the soil of the nitrogen assimilated before by the molds in an available form. The molds and the other organisms may act in the soil, from this point of view, as a storing agent for the soluble nitrogen compounds added to the soil, and the injury caused by them at times, in competing with the higher plants for the available nitrogen, may be more than balanced by their ability to store the nitrogen and make it afterwards slowly available for the plants. This subject requires further studies, particularly the question of symbiotic and antagonistic relationship between the higher plants and soil microorganisms.

#### ENZYME PRODUCTION BY MOLDS

A review of the literature on the enzyme production by molds can be found in the paper of Dox (14). Dox (14) found that *P. camemberti* contains the following enzymes: erepsin, nuclease, amidase, lipase, emulsin, amylase, inulase, raffinase, sucrase, maltase, and lactase; the protease digested casein, gelatin, and proteoses, yielding a large percentage of amino acids; the amidase liberated ammonia from urea, asparagin, benzamid, and alanin; another enzyme could split hippuric acid into benzoic acid and glycocholl. Kellerman (25) has demonstrated the production of a cellulose-splitting ferment by *Penicillium pinophilum*. Scales (49) demonstrated that *Aspergillus terricola* produced inulase, diastase, invertase, maltase, alcoholoxydase, emulsin, lipase, protease, and amidase. The production of proteolytic enzymes by molds was studied by the writer (61). The production of the enzymes enumerated by molds will bring forth the proposition that enzymes formed by soil molds as well as bacteria may be concerned in the decomposition of the various organic substances in the soil. Many of the products of this decomposition form good sources of nitrogen and carbon for bacteria, while the ammonia and some of the amino acids may be directly assimilated by higher plants.

#### THE POSSIBLE MODIFICATION OF THE SOIL REACTION BY THE ACTION OF MOLDS

It is a common belief among soil bacteriologists that the mold flora is more active in acid than in neutral or alkaline soils. The scant exact information that we have on this subject confirms this belief, although it does not preclude the growth of the molds just as well in neutral and perhaps alkaline media. The work of Thom and Currie (54) and Currie (10, 11) clearly demonstrate the fact that a number of *Penicillia* and *Aspergilli* produce a great deal of acid (citric and oxalic) due to the fermentation of cane sugar. Currie (10) found that different strains of *A. niger* and a certain *Penicillium* will grow at as high acidity as pH = 1.8-1.4, this point of acidity being produced by the use of hydrochloric, oxalic or citric acids. Very few of the soil molds have been studied for their acid production, but those used by the previously-named investigators were isolated by the writer from the soil. If not all

molds, then some of them at least are able to produce large quantities of acid from available carbohydrates. Currie found that a certain strain of *A. niger* can produce 10 to 12 per cent of citric and some oxalic acid in a 15 per cent cane sugar solution. If an assumption should be made that some of the soil molds are also as active in the production of acids from available carbohydrates, we might be able to account for at least some of the increasing acidity in soils and the necessity of lime to neutralize the acidity. It may be very possible that for a great deal of the soil acidity we should look not only to the production of mineral acids, due to the oxidation of minerals in the soil, or added fertilizers, but also to the organic acids, such as citric and oxalic, produced by soil molds as a result of the fermentation of the available carbohydrates. Hagem (20) found that several *Absidia* isolated from the soil produced a large quantity of oxalic acid, which, he thought, was probably due to the incomplete oxidation of glucose; the *Mucors* produced no oxalic acid, but another unidentified acid. These acids produced in the soil may have still another function: acting upon the insoluble phosphates and other minerals in the soil. They may thus bring about their transformation into a soluble form available for higher plants.

Kopeloff (29) found that a maximum ammonification by soil fungi was obtained between the neutral point and an acidity equivalent to 2,000 pounds of CaO per acre (Veitch method). These results point to a possibility that where the soil reaction may be unfavorable for the activities of the bacteria concerned in the decomposition of the organic matter, the molds might prove an important compensating factor in maintaining fertility. Hall and associates (21) stated that molds are the active agents in producing the acidity of the soil manured with ammonium salts. When ammonium chloride and sulfate are added to the soil, the basic ions are used up by molds, while the acid ions are left in the soil and are subsequently converted into hydrochloric or sulfuric acids.

#### THE EFFECT OF MOLDS UPON THE MINERAL TRANSFORMATIONS IN THE SOIL

The recent investigations on the mineral nutrition of fungi have been reviewed by Dox (15). The mineral nutrition of lower fungi and the other action of these organisms, direct and indirect, on the minerals of the soil, are still awaiting investigation. It is sufficient to mention that we know very little about the production of available phosphorus, oxidation of sulfur and iron in the soil and the production of available potassium from insoluble silicates, and particularly on the part played by molds in this respect. Results secured in this laboratory on the oxidation of sulfur by certain species of *Fusaria*, and the results of Brown and Corson (5) that *A. niger* is very active in the oxidation of iron in the soil, point to the fact that the molds probably are an important factor in these transformations.



## RELATION OF SOIL FUNGI TO PLANT DISEASES

It is known to plant pathologists that a soil may become sick with respect to a particular crop, due to the fact that yearly continuation of one crop on the same soil has introduced the organisms pathogenic to the particular crop into the soil, which therefore became a carrier for these disease-producing organisms. But parasitic fungi have been isolated also from virgin soils, or from soils on which the particular crop has never been grown before. Pratt (41) recently isolated fungi known to be parasitic on the Irish potato, from Idaho soils never cropped with potatoes and from virgin desert lands. We can also cite the work of Bolley (2) on the fungi parasitic to wheat isolated from the soil. More work is also needed on this subject, before we could definitely conclude how far the soil should be considered as a possible medium for facultative parasitic fungi.

## SUMMARY

1. Molds have been isolated in large numbers from different cultivated and uncultivated soils, and the identity of many genera and species isolated from widely different localities has been established. The cultivated soils contain by far a smaller number of molds than they do bacteria and actinomycetes.

2. Molds live and produce mycelium in the soil, and therefore take an active part in the transformation of some of the organic and inorganic substances, which are important factors in the fertility of the soil. The plate count of molds in the soil cannot be taken as an indication of the actual numbers of molds living in the soil.

3. The molds present in the soil, at least most of them, do not fix any atmospheric nitrogen, and even where fixation was shown to be positive, the quantities are so small as to be negligible in the study of soil fertility problems.

4. Molds do not seem to play any part in the process of nitrification.

5. The molds play an important rôle in the decomposition of organic matter with the subsequent liberation of ammonia. The amount of ammonia produced depends not only on the source of nitrogen, but also on the carbohydrates available.

6. The molds take an active part in the decomposition of the simple and complex carbohydrates in the soil, with the production of carbon dioxide; this brings about a mineralization of the organic matter which is thus made available for higher plants.

7. The molds utilize very readily the nitrogen compounds usually added to the soil in the form of different fertilizers and convert them into complex body proteins, thus competing with the green plants and exerting an injurious effect upon soil fertility. This may be somewhat counterbalanced by the fact that some of the soluble nitrogen compounds are thus saved from loss by drainage from the soil and that the fungus body undergoes autolysis thus liberating in a soluble form most of the nitrogen that it has assimilated.

8. The molds isolated from the soil produce a number of enzymes which may help to bring about decomposition processes which are important to the upkeep of the fertility of the soil.

9. The production of acids by some molds in the soil may account for some of the soil acidity and may help to dissolve the insoluble phosphates and other minerals necessary for the growth of the green plants.

10. A number of organisms parasitic to green plants have been isolated from soils, upon which these plants have often never been grown before.

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## THE EFFECT OF LIMING ON CROP YIELDS IN CYLINDER EXPERIMENTS

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In the spring of 1898 there was begun by the New Jersey Agricultural Experiment Station a series of cylinder experiments. It was proposed in carrying on these experiments to ascertain the significance, if any, of denitrification when moderate and fairly large amounts of nitrate of soda are used together with cow manure. It was also proposed to ascertain in these experiments the relative availability of nitrogen derived from nitrate of soda, sulfate of ammonia, dried blood and cow manure of varying composition. The experiments were carried out in triplicate, as shown in table 1. The complete experiment embraces 20 series of 3 cylinders each, but in this paper only 4 of the series are directly considered, viz: those that receive nitrogenous fertilizers in the form of nitrate of soda (series 7 and 8), sulfate of ammonia (series 17) and dried blood (series 18). A full report covering the first 15 years of this work has been published in Bulletins 221 and 288 of the New Jersey Agricultural Experiment Station, and a 20 years' summary of a part of the work in *Soil Science*, vol. 5, no. 4, April, 1918.

All the cylinders in the four series here considered have received annual applications of acid phosphate and muriate of potash equivalent to 640 and 320 pounds per acre, respectively. In addition to these minerals, nitrogenous fertilizers have been applied annually as follows:

Series 7, nitrate of soda at the rate of 160 pounds per acre.

Series 8, nitrate of soda at the rate of 320 pounds per acre.

Series 17, ammonium sulfate equivalent to 320 pounds of nitrate of soda per acre.

Series 18, dried blood equivalent to 320 pounds of nitrate of soda per acre.

All cylinders received a generous application of lime in the form of ground limestone at the time the work was started.

The crops were grown in a 5-year rotation and consisted of corn, 2 years of oats, wheat and timothy. In the period 1898 to 1907, inclusive, there were produced, therefore, two crops of corn, four crops of oats, two crops of wheat and two crops of timothy. In the period 1908 to 1917, inclusive, the same number of each of these crops were produced. In addition to these main crops, a residual crop of corn (millet in 1899) was planted after each oat crop for the purpose of more completely utilizing the nitrogen.

After the first 10-year period, viz.: in the spring of 1908, the original treatment was modified to the extent that the A cylinders in each series received no further additions of lime; the B and C cylinders in each series received a generous application of ground limestone once in each rotation, and in addition to the lime the C cylinders in each series were seeded to a leguminous green-manure crop—vetch and crimson clover—twice in each rotation.

These legumes were used to provide for increasing, from atmospheric sources, the supply of nitrogen for the crops grown in the C cylinders. Hence, the differentiation between the A, B and C cylinders introduced in 1908, consisted of modifying the soil reaction in the B cylinders and of modifying the soil reaction and increasing the supply of nitrogen in the C cylinders.

Comparing now the results secured in the 10-year period 1898 to 1907, inclusive, and the second 10-year period 1908 to 1917, inclusive, we find some very interesting differences as indicated by the data in table 1. It will be noted that during the first 10 years there were but slight differences in the yields of dry matter between the A, B and C cylinders in each series. Thus, the average yield of dry matter for the first 10-year period in series 7 was 199.48 gm. in the A cylinders, 210.93 gm. in the B cylinders and 201.28 gm. in the C cylinders. Relatively slight differences are found also in series 8, 17 and 18.

The results are quite different for the second 10-year period—1908 to 1917, inclusive. It will be noted that in series 7 the average for the A cylinders was 117.25 gm. The average for the B cylinders was 191.42 gm. and for the C cylinders 235.88 gm. Hence, the increase due to the use of lime in the B cylinders was from 117.25 to 191.42 gm. The further increase in the C cylinders should be attributed to the nitrogen introduced in these by the leguminous catch crops. Similar relations will be found to exist in series 8, 17 and 18. It is particularly interesting to note that in series 17 the soils in the A cylinders had become so acid as to have failed to produce any crop whatsoever in 1912, 1916 and 1917. On the other hand, in the B cylinders of the same series, the yield in 1917 was greater than that in 1907. To a less striking extent, soil acidity has become a very important limiting factor in the A cylinders of Series 18, where dried blood was used together with acid phosphate and muriate of potash.

Taking the averages for all of the A, B and C cylinders in the two 10-year periods, we note that, for the first 10-year period the A cylinders produced an average of 222.34 gm. of dry matter; the B cylinders, 223.07 gm. and C cylinders, 215.20 gm. of dry matter—amounts practically identical. On the contrary, the average for the second 10-year period was 128.87 gm. of dry matter in the A cylinders, 205.12 gm. in the B cylinders and 245.49 gm. in the C cylinders. There has, therefore, been a remarkable falling off in the yields of the A cylinders from the first to the second 10-year period. The yields in the B cylinders were practically maintained in the second 10-year period, while the yields in the C cylinders were actually increased from the first to the second 10-year period.

TABLE 1  
The effect of lime on crop yields (dry weight) in cylinder experiments

YEAR	SERIES 7			SERIES 8			SERIES 17			SERIES 18			AVERAGE		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1898	332.1	325.6	331.3	360.9	393.9	396.6	387.8	401.0	330.1	322.2	341.8	303.7	350.8	365.6	340.4
1899	172.8	182.0	183.1	207.9	184.5	221.4	192.9	190.5	172.5	177.8	186.3	176.0	187.9	185.8	188.3
1900	284.7	270.2	272.7	327.4	317.0	304.1	310.4	310.1	283.9	310.8	307.9	295.9	308.3	301.3	289.1
1901	226.6	317.9	187.9	339.2	331.0	317.1	298.7	300.0	257.6	276.1	239.4	291.5	285.2	297.1	263.5
1902	125.6	137.6	136.8	146.1	150.9	167.2	113.1	143.9	127.3	129.3	115.6	111.5	128.5	137.0	135.7
1903	186.0	189.0	215.0	255.0	183.0	245.0	317.0	291.0	251.0	235.0	216.0	225.0	248.3	219.8	234.0
1904	146.0	156.0	147.0	174.0	170.0	169.0	153.0	167.0	151.0	152.0	160.0	159.0	156.3	163.2	156.5
1905	180.0	182.0	176.0	224.0	226.0	218.0	207.0	209.0	190.0	218.0	191.0	174.0	207.3	202.0	189.5
1906	186.0	194.0	198.0	239.0	244.0	274.0	140.0	226.0	153.0	156.0	144.0	160.0	180.3	202.0	196.2
1907	155.0	155.0	165.0	206.0	168.0	194.0	150.0	133.0	99.0	172.0	172.0	177.0	170.7	157.0	158.8
Average first 10 years. ....	199.5	210.9	201.3	247.9	236.8	250.6	227.0	237.2	201.5	214.9	207.4	207.4	222.4	223.1	215.2
1908	188.0	228.0	249.0	257.0	331.0	332.0	239.0	286.0	291.0	182.0	228.0	245.0	216.5	268.3	279.2
1909	164.0	198.0	228.0	189.0	244.0	245.0	142.0	217.0	215.0	169.0	218.0	235.0	166.0	219.2	230.8
1910	220.0	273.0	367.0	312.0	338.0	460.0	202.0	287.0	366.0	217.0	276.0	353.0	237.8	293.5	386.5
1911	90.0	116.0	107.0	147.0	160.0	153.0	83.0	117.0	128.0	141.0	126.0	147.0	115.2	129.8	133.8
1912	43.0	119.0	155.0	99.0	187.0	182.0	00.0	153.0	147.0	52.5	115.0	137.0	48.6	143.5	155.2
1913	195.9	274.0	271.5	253.0	312.5	324.9	239.7	228.5	280.7	234.2	286.5	277.5	230.7	275.4	288.7
1914	141.4	196.2	353.8	172.0	222.4	341.1	132.7	196.9	333.0	164.0	198.3	370.2	152.5	203.5	349.6
1915	91.7	187.4	281.3	100.5	211.0	268.2	64.8	178.3	254.3	103.2	147.5	243.0	90.0	181.0	261.7
1916	20.5	169.4	147.2	24.0	217.3	225.4	00.0	181.6	170.4	4.0	112.9	149.1	12.1	170.3	173.0
1917	18.0	153.2	199.0	29.0	208.0	232.3	00.0	167.0	175.1	29.5	139.0	179.3	19.1	166.8	196.4
Average second 10 years. ....	117.3	191.4	235.9	158.3	243.1	276.4	110.3	201.2	236.1	129.6	184.7	233.6	128.9	205.1	245.5



In considering the data presented in table 1, we may readily understand that the acid residue from ammonium sulfate would interfere with normal plant growth where such residues are accumulated in considerable quantities. But in the case of nitrate of soda we might have expected that the basic residues would, to some extent at least, retard the accumulation of soil acidity. We note, however, that in series 7, as well as in series 8, the yields in the B cylinders were very much larger than those in the corresponding A cylinders. This would tend to show that, where commercial fertilizer alone is used as a source of plant-food and in amounts corresponding to those employed in the present experiments, there would be a very marked accumulation of soil acidity, and a very marked improvement in plant growth after the use of adequate quantities of lime. The most striking differences in yields between the A cylinders and the corresponding B cylinders occurred, of course, in series 17. Here the average yield in the A cylinders was 226.99 gm. and in the B cylinders 237.15 gm. for the first 10-year period. For the second 10-year period, the corresponding yields were 110.32 gm. and 201.23 gm., respectively. Even in series 8 where nitrate of soda was used at the rate of 320 pounds per acre, the basic residues have not been sufficient to keep the soil in a good condition for a long period of years, as is shown by an average of 247.95 gm. for the first 10 years in the A cylinders—unlimed—and average of 158.25 gm. for the second 10 years. On the other hand, the B cylinders of this series—limed—gave an average of 236.83 gm. for the first 10 years and 243.12 for the second 10 years. It is quite apparent, therefore, that the continued use of acid phosphate, muriate of potash, nitrate of soda, sulfate of ammonia and dried blood, in amounts corresponding to those employed in the experiments described here, is bound to lead, sooner or later, to an unsatisfactory soil reaction and to the need of generous applications of lime. Indeed, the writers are convinced that sufficient stress is not laid on the importance of systematic and adequate liming of land whose production is to be brought up to constantly higher levels by the generous use of commercial fertilizers.

Emphasis is also laid on the importance of introducing leguminous crops in the rotation at frequent intervals for the purpose of increasing the supply of *available* nitrogen and also to maintain a good supply of organic matter.

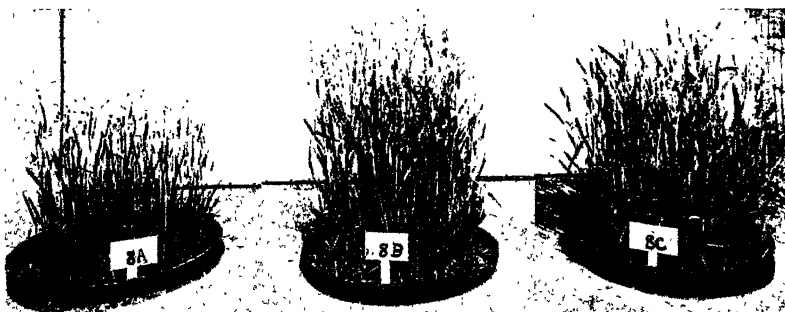


Fig. 1. Fertilizer treatment: 10 gm. nitrate of soda, 20 gm. acid phosphate, and 10 gm. muriate of potash per cylinder. A no lime; B lime; C lime and green manure.



Fig. 2. Fertilizer treatment: sulfate of ammonia equivalent to 10 gm. nitrate of soda, 20 gm. acid phosphate and 10 gm. muriate per cylinder. Lime and green-manure treatment as in figure 1.

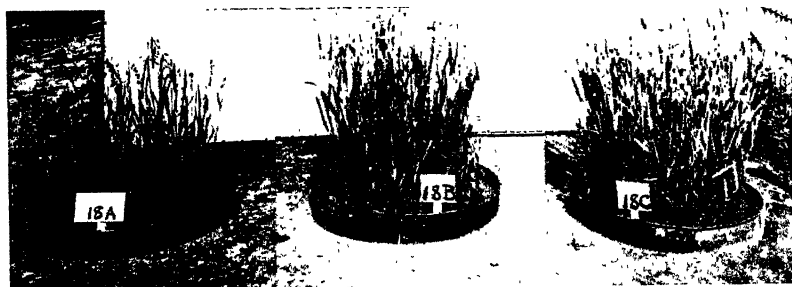


Fig. 3. Fertilizer treatment: dried blood equivalent to 10 gm. nitrate of soda, 20 gm. acid phosphate and 10 gm. muriate of potash per cylinder. Lime and green-manure treatment as in figure 1.



## AZOFICATION

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The maintenance of the nitrogen supply of the soil is the phase of soil fertility which has received greatest consideration both from the scientist and from the practical agriculturist. Nitrogen is one of the more expensive commercial fertilizers and is, in the majority of soils, the limiting factor of crop production. The supply of combined nitrogen on the earth is comparatively small and it is possible to calculate approximately the time necessary for its exhaustion. Basing his conclusion on such a calculation, at least one scientist has predicted dire calamity to the human race were science not able soon to solve this problem. Science has measured up to its requirements in this regard, for the synthetic production of combined nitrogen has been accomplished, and this in a manner so highly satisfactory that it is able to compete successfully with the product of natural deposits. Advancements have also been made in our knowledge of the underlying principles influencing the natural processes which govern the fixation of nitrogen in the soil. Although there is much yet to be learned in this field it is upon the control of these natural processes that ultimate success will be based.

It has been known for generations that uncropped soils increase in fertility. Less ancient, however, is the knowledge that this increase may be due to a gain of nitrogen in the abandoned soils. Even more recent than this is the knowledge that it may be due to bacteriological action.

In the middle of the nineteenth century Boussingault (19) wrote: "Vegetable earth contains living organisms—germs—the vitality of which is suspended by drying and reestablished under favorable conditions as to moisture and temperature." He also hinted at the fact that these microorganisms take part in the process of nitrogen fixation. He spread out thinly 120 gm. of soil in a shallow glass dish and for three months moistened it daily with water free from nitrogen compounds. At the end of this time analysis showed that it had lost carbon, but had gained nitrogen. It was not until thirty years later that Hellriegel and Wilfarth made their discovery of nitrogen-fixation by symbiotic organisms. At that time the laboratory technique of modern bacteriology was still undeveloped. Since then, however, we have learned much concerning the relationship of plants to free and combined nitrogen of the air and of the soil. We know that soil gains in nitrogen are often due to microorganisms, either living free in the soil or in company with the

higher plants. The production of nitrogen compounds out of atmospheric nitrogen by bacteria independent of higher plants is designated non-symbiotic nitrogen-fixation, or azofication. When fixation is accomplished by bacteria living in connection with and receiving benefit from higher plants, it is considered symbiotic nitrogen-fixation.

As early as 1883 Berthelot (13) undertook the study of soils with regard to their relation to free and combined nitrogen, and as a result of these studies he was the first definitely to recognize that gains which occur in bare unsterilized soils are due to microscopic organisms. He found that when 50 kgm. of arable soil were exposed to air and to rain in a vessel for 7 months, after allowing for the small amount of combined nitrogen brought down by the rain, there was a gain in nitrogen of more than 25 per cent. In another experiment in which the soil was first washed free from nitrates, there was a gain of 46 per cent. Many other experiments showed gains from 10 to 15 per cent. Berthelot was not content with the bare knowledge that nitrogen is fixed in the soil by living organisms, but continued his work with the idea of isolating some of these organisms. With the aid of Guignard, he made soil inoculation into sterile bouillon and from this prepared gelatin plates. Cultures were taken from the colonies growing on the plates and bacteria were tested for their nitrogen-fixing power. His results were conclusive that there exist within the soil chlorophyll-less bacteria capable of fixing atmospheric nitrogen. His work had shown that these organisms act best at summer temperatures, between 50° and 104° F., in the presence of a good supply of oxygen, a proportion of water in the soil not exceeding 12 to 15 per cent and not falling below 2 to 3 per cent. They require carbon, hydrogen and enough combined nitrogen to promote initial growth. The nitrogen, gained by the soil was proteinaceous in nature, being insoluble in water. Although some of his soils had gained large quantities of nitrogen, he considered that the fixation of atmospheric nitrogen by microorganisms has its limits, since the organisms isolated drew from the atmosphere only so long as the amount fixed in the medium was not great. Heating the soil to 230° F. immediately stopped the process.

Prior to this a number of chemists, notably König and Kiesow (101), Armsby (1), Birner (14), Kellner (91), Deherain (35) and Avery (3) had found that when organic matter in one form or another undergoes fermentation there is frequently an increase of nitrogen in the fermenting substance. Armsby states it thus: "We must conclude that decaying organic substances in the presence of caustic alkali are able to fix free nitrogen without the gain being manifest as nitric acid or ammonia, and probably with the formation of these bodies." His explanation of the process was that the nascent hydrogen evolved during the fermentation process reacted with the free nitrogen of the air. Others considered that the active agents were compounds of iron, manganese, and lime existing in the soil and in some way acting as catalytic agents.

Berthelot's discovery interested Winogradski (209) who commenced work which eventually bridged the chasm. He employed, as a medium, a nutritive solution free from combined nitrogen, but containing mineral salts and dextrose. Fifteen separate species of soil bacteria were isolated, but only one—a long spore-bearing bacillus which developed normally in the absence of combined nitrogen and seemed to produce butyric fermentation—fixed nitrogen to any appreciable degree. Quantitative tests showed that the maximum fixation was attained where no combined nitrogen was purposely added, and that on the addition of such, fixation of nitrogen was diminished. For example, several determinations gave the following results:

N as $\text{NH}_3$ in dextrose solution.....	2.1	4.2	6.4	8.5	21.2
N fixed.....	7.0	5.0	5.5	3.6	2.2

The presence of combined nitrogen tends to decrease fixation. He concluded that in order for any gain to be made, the ratio of the combined nitrogen to the sugar should not exceed 6:1000. Because of the characteristic formation of clostridia in his cultures, Winogradski named the organism *Clostridium pasteurianum*. The conclusion which the author reached, however, was that the power of fixing nitrogen is not general among microorganisms, but confined to a few special forms.

Following Winogradski, Caron (25) made some very interesting discoveries. He found that soils under leafy crops contain greater numbers of bacteria than those under grasses. He also observed that the bacterial flora of soils in the spring are different from those in the fall both quantitatively and qualitatively. He used in vegetation experiments pure cultures of the bacteria most frequently encountered in natural soils. Some soils were inoculated with bouillon culture, whereas others received only sterile bouillon. The crop yields were usually in favor of the inoculated plots, but showed variations from season to season. Exceptionally good results were obtained with a spore-bearing bacillus which he termed *Bacillus ellenbachensis*.

Caron's work led to the commercial exploitation of his cultures, one of which, "alinit," was the subject of much study and discussion. This culture was found to contain, according to Severin, two closely-related bacilli which he chose to designate as *B. ellenbachensis* A and B. These had the power to fix nitrogen to some extent. Tests with "alinit," however, have not confirmed to any great extent the claims of its exploiters.

In 1901 Beijerinck's (7) investigations led to an extremely important addition to the history of non-symbiotic nitrogen-fixation. He described a new group of large aerobic bacilli to which he gave the generic name *Azotobacter*.

In an early paper published by Beijerinck and Van Delden (9), they maintain that *Azotobacter* are incapable of fixing appreciable quantities of nitrogen in pure culture, but are dependent to a large extent on *Granulobacter*, *Radiobacter*, and *Aerobacter*. They considered that in mixed cultures the *Granulobacter*, *Radiobacter*, and *Aerobacter* possess the power of fixing nitrogen in the

presence of *Azotobacter*, which grows at the expense of the combined nitrogen escaping from them into the solution.

A little later Gerlach and Vogel (50) succeeded in isolating from soil the *Azotobacter* of Beijerinck and in showing that in pure cultures and in the presence of salts of organic acids, *Azotobacter* are capable of active nitrogen-fixation. They obtained a fixation of 9 mgm. of nitrogen in a 1 per cent solution of grape sugar. But Beijerinck challenged this assertion claiming that their cultures were not pure but were mixed with other forms difficult to separate. The claims of Gerlach and Vogel were substantiated by the work of Freudenreich (47), Koch and Lipman (122). The latter not only showed that the *Azotobacter* possess the power of fixing nitrogen in pure cultures, but he explained the failures recorded by others.

Although not necessary, the presence of other organisms often proves advantageous (47). Lipman (122) found that in the presence of such forms as *B. radiobacter* and *B. levaniformis* the nitrogen-fixation is faster and goes on at a more regular rate.

To the two species of *Azotobacter*—*A. chroococcum* and *A. agilis*—described by Beijerinck and Van Delden, Lipman (122, 123) added *A. vinelandii*, *A. beijerinckii*, and *A. woodstownii*. Later Löhnis and Westermann (134) described *A. vitreum*, and after a study of 21 cultures of various *Azotobacter* concluded that they represented only four types. *A. chroococcum* is most widely distributed in the soils so far studied.

The discussion of the subject thus far has been more or less confined to the *Azotobacter*, but investigations of Beijerinck and Van Delden (9), Löhnis (127), Moore (144), Chester (26), Bredemann (20) and others (168) have brought to light other microorganisms having the power to fix nitrogen. Among these are *B. mesentericus* (which fixes appreciable quantities of nitrogen), *B. pneumoniae*, *B. lactis viscusus*, *B. radiobacter*, *B. prodigiosus*, *B. asterosporus* and *B. amylobacter*.

Bredemann (20), after a careful study of the morphological and physiological characteristics of eleven "original species" of other investigators and of sixteen cultures prepared by himself from various soils, concluded that all belong to the single species *B. amylobacter* of Van Tiegham. Since this, however, there has been described at least one aerobic (168) clostridium. Moreover, Omelianskii (148) considers that the *Clostridium pasteurianum*, isolated from the Russian soils, is clearly a morphologically distinct race. An idea of the activity of some organisms in fixing nitrogen may be obtained from the following results reported by Löhnis (128). In every 100 cc. of 1 per cent mannite, or grape sugar soil extract, there was fixed, in the course of 3 weeks, nitrogen as follows:

	mgm.		mgm.
<i>Microc. sulfursus</i> .....	2.8 to 3.0	<i>Bact. chrysogloea</i> .....	1.4
<i>Bact. prodigiosus</i> .....	0.7 to 1.8	<i>Bact. tataricus</i> .....	0.3
<i>Bact. turcosus</i> .....	0.3 to 1.6	<i>Bact. lipsiense</i> .....	0.2

C. B. Lipman (114) tested 18 organisms, including yeasts, pseudo-yeasts, and molds, nearly all of which showed a more or less pronounced power of fixing atmospheric nitrogen.

Pringsheim (159) has isolated from ordinary garden soil certain thermophilic organisms which fix from 3 to 6 mgm. of nitrogen per gram of dextrose when incubated at 61°C. in a Winogradsky's solution to which a little soil extract was added. Duggar and Davis (36) have recently investigated the subject of the fixation of nitrogen by the filamentous fungi, *Aspergillus niger*, *Macrosporium commune*, *Penicillium digitatum*, *Pexpansum*, *Glomerella*, *Gossypii*, and *Phoma betæ*; and of these only the last-named was definitely proved to be able to fix nitrogen. It is thus seen that the power of fixing nitrogen is a characteristic possessed by many microorganisms, in contradiction to the supposition of Winogradsky that this power is limited to a particular, or, at most, a few species. This is especially emphasized by the recent work of Emerson (39) who examined soil which contained 2,400,000 organisms which would develop on nitrogen-free media. Of these, 97 per cent possessed the power of fixing nitrogen; they constituted at least four distinct groups. Nevertheless, the most important group yet discovered is the *Azotobacter*, and it is with these mainly that this paper deals.

#### DISTRIBUTION

The nitrogen-fixing organisms are widely distributed, occurring in most soils. Lipman and Burgess (117), who studied the nitrogen-fixing flora, especially those of the *Azotobacter* group, of 46 soils from Egypt, India, Japan, China, Syria, the Hawaiian Islands, Guatemala, Costa Rica, Spain, Italy, Russia, Mexico, Asia Minor, Canada, Unalaska, Samoa, Australia, Tahiti, Belgium, Queensland, and the Galapagos Islands, found every soil possessed the power of fixing nitrogen in mannite solution. About one-third of the soils contained *Azotobacter*; frequently the same soil showed the presence of two or three different species of *Azotobacter*. *A. chroococcum*, however, was the most prominent. It was also found most widely distributed in the various soils. Groenewege (62) found *Azotobacter* in all but one of a series of Java soils.

Several hundred Utah soils have been examined and all found to fix nitrogen (55), many of them without the addition of carbohydrates. Aerobic *Azotobacter* are present in nearly all Utah soils. Hutchinson (85) found the *Azotobacter* in all the Indian soils examined. They occur in cultivated more frequently and in greater numbers than in virgin soils. This probably accounts for the much higher nitrogen-fixing power of cultivated soils.

*Azotobacter* were found in only two out of 64 localities in the soils of Danish forests (204). Both of the soils which gave positive tests were from beechwood forests and contained calcium carbonates. Although the soils of these forests rarely contain enough carbonate to effervesce, they are usually neutral



or slightly alkaline. They contain calcium, but in forms other than the carbonate. It is generally understood that *Azotobacter* occur commonly in soils which contain sufficient calcium carbonate to effervesce when acid is added and that they scarcely ever occur in acid soils. Their disappearance from a soil is usually due to the absence of basic substances, especially of calcium and magnesium carbonate, and not to the presence of toxic substances (28). However, they are frequently not present in peaty soils, where their absence cannot be attributed to a lack of lime (41).

The aerobic nitrogen-fixers are probably more widely distributed in soils than are the anaerobic, for, although both groups are generally found in the Russian soils (148), the aerobic are found in the sands of Kirghese steppes and in the peat soils of the Province of Archangel in which the anaerobic forms are absent. Anaerobic nitrogen-fixers are, however, quite widely distributed in soils and are at times found on the leaves of forest trees (68).

The nitrogen-fixing organisms are confined almost entirely to the first three feet of soil (115), although they have been found in soil at all depths down to the tenth foot in the very favorable constituted loess soils of Nebraska (200).

They are most active in the upper few inches of soil, as is indicated by results obtained by Ashby (2).

SOIL	DEPTH	AVERAGE NITROGEN FIXED
	cm.	mgm.
Little Hoos .....	10	9.23
Little Hoos .....	20	7.29
Little Hoos .....	30	4.60

Reports on some Hawaiian soils (150) show them to be equally active at all depths to 4 feet, but this must be considered an exception, for the examination of numerous soils in Utah (57) has shown a gradual decrease in nitrogen-fixing powers with depth. The average of several hundred determinations, in both solution and soil media, are given below:

DEPTH OF SAMPLE	NITROGEN FIXED IN 100 GM. OF SOIL + 1.5 GM. OF MANNITE	NITROGEN FIXED IN 100 CC. OF ASHBY'S SOLUTION WITH 1.5 GM. OF MANNITE
	mgm.	mgm.
First foot.....	5.28	2.11
Second foot.....	2.42	0.77
Third foot.....	1.55	0.58

These samples were collected with such great care that there was no possibility of the mixing of one foot section with another. It is interesting to note that while the actual gain in nitrogen per gram of mannite is over twice as

great in the soil as in the solution, yet the relative gain per foot section is the same in both. There is about one-half as much nitrogen fixed in the second as in the first foot, and one-fourth as much in the third as in the first.

The nitrogen-fixing organisms are not confined to the soil alone, for Beijerinck and Van Delden first isolated *Azotobacter agilis* from canal water in Holland (9). *Azotobacter chroococcum* and *B. Clostridium pasteurianum* are both found in many fresh and salt waters (11), living on algæ and plankton organism (10).

#### REACTION OF THE MEDIA

The distribution and the physiological efficiency of the nitrogen-fixing organisms, especially of the *Azotobacter* species, are governed by the physical and chemical properties of the soil, foremost among which is the basicity of the soil, namely, its calcium or magnesium carbonate content (27). Ashby (1) bases his method for obtaining pure cultures of *Azotobacter* upon this property, for he finds that by picking out the crystals of the carbonate from the soil and seeding them into nitrogen-free media the likelihood of obtaining the organism is greatly increased. The addition of calcium carbonate to a soil often increases its azofying power (6), the extent of which increase depends on the lime requirements of the soil and on the fineness of the added limestone (102).

Christensen (28) has suggested that the *Azotobacter* be used as an index to the lime requirements of a soil. The test should include both a search for the organism in the soil and a test of their ability to grow when inoculated into the soil. He and Larson (29) examined more than one hundred soils of known lime requirement. They determined the carbon dioxide set free by acids, the amount of calcium dissolved by an ammonium chloride solution, the behavior of the soil toward litmus, and the biological test. The result of this test was that the biological test agreed with the known condition in 90 per cent of the cases, the ammonium chloride in 50 per cent, the litmus in 40 per cent, and the carbon dioxide failed more often than not to indicate the correct condition of the soil.

Fischer (43) failed to find *Azotobacter* in a heavy loam soil containing only 0.145 per cent of lime, while adjoining limed plots had an *Azotobacter* flora. The quantity of calcium carbonate which must be added to obtain maximum fixation varies with the soil (81).

A West Virginia Dekalb silt loam (6), which required 0.175 per cent of calcium carbonate to render it neutral by the Veitch method, gave greatest nitrogen fixation when 0.375 per cent of calcium carbonate was added. Above this concentration azofication decreased, but when phosphorus was applied with the lime it was not toxic even when present in quantities as great as 0.5 per cent. It is certain that large quantities of calcium carbonate may be present in soil without injury to the azofiers (139).

The author found numerous *Azotobacter* and a very active nitrogen-fixation in a soil 43 per cent of which was calcium and magnesium carbonate (59).

The organisms develop normally in the presence of either calcium or magnesium carbonate, but in liquid cultures the film develops earlier and it contains less foreign organism in the presence of magnesium carbonate than in the presence of calcium carbonate. The actual nitrogen fixed, as reported by Ashby (1), is also greater where the magnesium carbonate is used. This he attributes to the suppression by the magnesium of foreign organisms, especially of the butyric acid ferments.

There is, however, a marked difference in the action of calcium carbonate and magnesium carbonate when they are applied in large quantities. Lipman and Burgess (116) found the calcium carbonate stimulating and never toxic to *Azotobacter chroococcum* in concentrations up to 2 per cent in mannite solution. The magnesium carbonate was sharply toxic in higher concentrations above 0.1 to 0.2 per cent in such cultures. The calcium salt is without effect when added to most soils up to 1.4 per cent, but the magnesium carbonate is even more toxic in soils than in solutions. Moreover, their work indicates that calcium exerts a protective influence, in both soils and solutions, against the toxic influence of magnesium. The best ratio of calcium to magnesium varies with solution and soil.

In many soils lime increases the nitrogen fixed, for Krzemeniewski (113) found limed soil to fix in 10 days 17.52 mgm. of nitrogen, whereas adjoining unlimed soil fixed only 7.15 mgm. There is, however, the possibility of applying too large a quantity of the caustic lime and thereby decreasing nitrogen-fixation (95), a condition which has never been experienced in the use of the carbonate.

Von Feilitzen (41), however, found neither a direct relationship between lime content of moor soil and the development of *Azotobacter*, nor relationship between their development and the reaction of the soil. But this only serves to illustrate the fact that although lime and neutral or slightly alkaline media are essential, they will not insure a rich *Azotobacter* flora in a soil unless all other conditions are optimum. Remy (165) found sodium and potassium carbonate less favorable for nitrogen-fixation than were calcium or magnesium.

So far as the writer is aware, Krainskii (105) is the only worker who has found sodium carbonate more favorable than calcium carbonate. This may have been due to the sodium carbonate's liberating plant-food which was in the soil in an insoluble form but which was essential to the development of *Azotobacter*. Mockridge (139) has found that the presence of sodium salts is unnecessary and depressing at least to the growth of *Azotobacter*. The beneficial effect ascribed to sodium chloride solution in inoculating agar plates is due to the fact that this liquid is isotonic with the cell content solution, but the sodium hydroxide is a far less advantageous neutralizing agent than is calcium or magnesium carbonate (139). Furthermore, Lipman failed to stimulate the azofiers with any of the sodium salts.

## FOOD REQUIREMENTS OF THE AZOFIERS

These organisms probably require for their nutrition the same elements as do the higher plants, namely carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, sulfur, calcium, magnesium, and iron, and possibly aluminum and manganese.

They obtain their carbon and hydrogen from organic compounds, preferably from carbohydrates, which are considered in detail under sources of energy. Oxygen is obtained either from the atmosphere or from combined sources depending on the species and the conditions under which they are grown.

A marked difference between these and the higher plants is that they possess the power of obtaining their nitrogen from the air, but in the presence of combined nitrogen they obtain but little from the air (191). Lipman (122), Stranak (191), Heinze (74), and Stoklasa (187) found that small quantities of nitrates stimulated *Azotobacter*, whereas large quantities discouraged nitrogen-fixation since the organisms live on the nitrates. This is the case whether the nitrates are added to the soil or to the solution in which nitrogen-fixation is taking place. Coleman (30) considers this action as due to several different factors: namely, (a) a direct toxic action of the salt, (b) antagonism of other organisms which it favors, (c) the using up of the energy supply by these organisms, and (d) the discouragement of fixation by the use of sodium nitrate. The last would seem to be the most important factor when viewed in connection with the following results reported by Hills (77):

TREATMENT NITRATE	RELATIVE NUMBER OF ORGANISMS			RELATIVE PER CENT OF NITROGEN FIXED			
				Sterilized soil		Unsterilized soil	
	KNO <sub>3</sub>	NaNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	NaNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	NaNO <sub>3</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>
mgm.							
0	100	100	100	100	100	100	100
10	348	191	362	100	105	240	219
50	8,210	3,150	4,528	342	371	500	444
150	12	117	763				
200	0	0	0	352	467	879	557

The number of organisms developing and the nitrogen fixed in the one receiving no nitrate is taken as 100 per cent.

It is quite evident from these results that although nitrates cause more active multiplications of *Azotobacter*, it greatly reduces their physiological efficiency. The organisms used by Hills had probably grown for a long time on media poor in nitrogen, and their ability to fix nitrogen was, therefore, high. But would they continue to exert this power if grown on media rich in nitrogen? The evidence points strongly to the conclusion that they

would not. It is certain, however, that the nitrates are toxic in comparatively low concentrations. Nitrates and ammonium sulfate are rather effective in stimulating nitrogen-fixation when the *Azotobacter* are grown in connection with the cellulose ferments (136). Even here, however, large quantities decrease this power. In pure cultures ammonium sulfate (108, 122) seriously retards nitrogen-fixation, whereas the nitrogen of humus, even in large quantities, appears to have no serious retarding influence (65). Nevertheless, a high nitrogen content of soils seems to be unfavorable to vigorous nitrogen-fixation (117). Whether this would be the case where the nitrate content of the soils is kept low but with the readily-decomposable protein nitrogen high is yet to be answered. Hiltner and Störmer (79) consider that when the nitrogen content of the soil passes beyond a certain limit, the decay bacteria increase rapidly, and in the struggle for existence they are able, with the advantage at their disposal, to suppress the more slowly growing *Azotobacter*.

Potassium is essential to the higher plants and cannot be replaced entirely by related substances, yet Gerlach and Vogel (50, 51, 52) early reached the conclusion that potassium and magnesium are not essential to the *Azotobacter*. Their results were, however, generally considered erroneous, for while as much nitrogen was fixed in 20 days without as with potassium, after 40 days there was no further fixation in the solution without potassium, but in its presence the nitrogen gain nearly doubled. It was, therefore, argued that the traces of potassium left in the chemicals and dissolved from the glass during sterilization had been enough to permit development for a time. If these elements are essential, it must be in extremely minute quantities, for Vogel (197), using the purest chemicals obtainable, was able to prepare potassium-free media in which the *Azotobacter* developed. He did find, however, that potassium favors their development.

Phosphorus is required by these organisms (72, 206), large quantities being used for the building of the nucleo-proteins and phospho-proteins in which their bodies are extremely rich. Moreover, it greatly accelerates the reaction and economizes the carbohydrates; hence it is rather evident that phosphorus plays a very essential part in *Azotobacter* metabolism. Possibly in the early stages of the process a definite chemical reaction occurs between the phosphate and the carbohydrate similar to that occurring in alcoholic fermentation (67).



The *Azotobacter* are able (199) to utilize the phosphorus of di- and tri-basic sodium and potassium phosphate and of dibasic calcium phosphate (98). Mockridge (139) obtained an increase of 23 per cent in nitrogen-fixation with basic slag. There were two maxima, one with 0.4 per cent, the other with 1.0 per cent slag. This is attributed to the stimulating effect of the iron and

manganese in the slag, the maximum effect of one being produced at 0.4 per cent, the other at 1.0 per cent. The tribasic calcium phosphate, bone ash, iron and aluminum phosphate all serve only as difficultly available sources of phosphorus. Raw rock phosphate and bone meal fail entirely to furnish enough available phosphorus for the development of *Azotobacter* (27).

The addition of phosphorus to a soil often greatly increases azofication (6).

TREATMENT	WITHOUT P	WITH P
	mgm.	mgm.
No lime.....	0.6	0.9
Lime.....	1.5	4.6

Moreover, Christensen (27) has found soils in which phosphorus is the limiting element in *Azotobacter* growth. He entertains the hope that in view of the relationship between *Azotobacter* growth and lime and phosphorus that it will become eventually possible by the determination of bacterial food requirements to secure a general expression for the soil content of plant-food available to crops. He (28) further suggests that where a mannitol solution free from phosphorus produces a vigorous growth of *Azotobacter* after inoculation with a soil, it may be assumed that the soil is not deficient in available phosphorus. Dzierzbicki (38) notes that if soils are deficient in available lime, phosphoric acid, or potash, nitrogen-fixing bacteria such as *Azotobacter* are either entirely absent or present only in small numbers.

There is a definite relationship between the carbon and phosphorus content of a soil and the nitrogen assimilated. According to Stoklasa (189) *Azotobacter* assimilates from 5.0 to 5.7 gm. of free nitrogen for every gram of phosphorus used. Although these organisms are directly dependent upon a readily-available supply of phosphorus to promote growth, they do not change it into the organic form as rapidly as do the ammonifying bacteria.

Sulfur is required by the azofiers possibly for the formation of the proteinaceous material of their bodies. It is certain that the benefit derived by *Azotobacter* from the sulfates of iron and calcium is due in a large measure to the sulfur which these compounds supply. No evidence has as yet been produced which would lead us to believe that the organisms can use sulfur as a source of energy.

Calcium carbonate and calcium oxide, in addition to furnishing a base which neutralizes the acid formed in the metabolic processes of the *Azotobacter*, also furnish calcium to the organism. Christensen (27) brought out the fact that *Azotobacter* can derive their calcium from dibasic calcium phosphate and some calcium salts of organic acids. They could not, however, utilize the calcium of tribasic phosphate, of calcium chloride or sulfate.

Iron (95) is essential and either the ferric or ferrous sulfate is especially beneficial (98). Rosing (169) found the amount of nitrogen fixed increased

from 2.23 mgm. to 10.3 mgm. per gram of mannite when iron sulfate was added to the cultural media. This is due, in a great degree, to the iron which serves as food for the organism, yet its colloidal nature may play a part, for both organic and inorganic colloidal substances have an especially favorable action on *Azotobacter*, although the action of the inorganic colloids is fully manifest only in the presence of organic colloids (155). If used alone, large quantities of the ferric hydroxide are essential for the maximum effect, but in the presence of organic colloids, very small quantities of iron are effective. This has been attributed to the action of the colloidal iron which absorbs the nitrogen and oxygen of the air and brings them into more intimate contact with the *Azotobacter* (178). This would not only accelerate the normal processes of the aerobic *Azotobacter* by furnishing them with nitrogen and oxygen but it would tend to suppress the anaerobic processes which are extremely wasteful of the food. According to Kaserar (88), these organisms also require aluminum. Although this may accelerate, it has not been proved to be essential to their growth.

While not essential to the organisms, manganese is an extremely active catalyzer (61) in increasing proportions up to 6 mgm. per 100 cc. of media. Above this concentration the reaction falls off rapidly, and at 20 mgm. it is less than in the absence of manganese. It is oxidized by *Azotobacter*, and in the proportion of 1 part to 200,000 parts of soil it is an active stimulant. Olaru (146) considers it likely that the increased yield obtained after the application of manganese compounds to a soil is due to its accelerating the action of the nitrogen-fixing organisms of the soil.

#### ALKALI SALTS

In addition to the essential elements of plant-food applied to a soil, other so-called soil amendments are often added. These may influence the physical, chemical, or bacterial properties of the soil. Some substances may alter the physical properties of the soil to such an extent that the bacterial flora is modified. Others may react chemically with constituents within the soil and in so doing liberate substances which can be utilized by the bacteria. Again, there may be a direct stimulation or retarding effect upon the organisms. Within this field there is much yet to be learned concerning the nitrogen-fixing organisms. We have, however, some information concerning the influence of the so-called alkalies upon the nitrogen-fixing organisms. A large number of analyses have shown that sodium salts are not necessary for the activity of the *Azotobacter* (139), nor are they stimulated by the common soil "alkalies—sodium chloride, sodium sulfate or sodium carbonate" (118). In this latter respect they differ greatly from the ammonifying and nitrifying organisms.

They are, however, quite resistant to these compounds, as may be seen from the following reported by Barnes and Ali (4).

Nitrogen fixed per gm. of mannite in nutritive solution inoculated with salt land.....	1.23
Nitrogen fixed per gm. of mannite in nutritive solution inoculated with sterile soil.....	7.80
Nitrogen fixed per gm. of mannite in nutritive solution inoculated with normal soil.....	7.07

Soil which contained sufficient salt to check all vegetation contained nitrogen-fixing organisms. Barnes and Ali hold that salts do not accumulate in the soil in sufficient quantities to kill the nitrogen-fixing organisms, but they are rendered inactive and as soon as the salts are leached from the soil the *Azotobacter* commences to work. Keutner (92) who worked with marine forms of the azofiers, found they would grow and assimilate nitrogen in an 8 per cent solution of sodium chloride. Nitrogen-fixers growing in arable soil would not be as resistant as are those which have become adapted to a medium with a high osmotic pressure, but *Azotobacter* in general appear to be more resistant to alkali salts than are most other soil organisms, for no toxic influence was noted by Lipman (118) until the concentration of sodium chloride in the soil reached 0.5 per cent, sodium sulfate 1.25 per cent, and sodium carbonate 0.4 per cent. They are much more sensitive to sodium in the form of nitrates, for 0.15 per cent stopped their multiplication and probably killed many of them (77).

#### NON-VOLATILE ANTISEPTICS

Arsenic, lead, copper, etc., when applied to soil in the form of lead arsenate, sodium arsenate, arsenic trisulfid, or zinc arsenite, stimulate azofication (56). This is greatest with lead arsenate and least with zinc arsenite. Paris green not only does not stimulate, but is toxic when the concentration reaches 120 parts per million. The toxicity, however, is due to the copper and not to the arsenic contained in it. Sodium arsenate becomes toxic when a concentration of 40 parts per million of arsenic is added, and when 250 parts per million are added it entirely stops nitrogen fixation. Lead arsenate is not toxic even at a concentration of 400 parts per million of arsenic. The toxicity of arsenic trisulfid and zinc arsenite is only slight at this concentration.

The stimulation occurring when arsenic is added to a soil is not due to any inherent peculiarity of one soil, for soils which differ greatly in physical and chemical properties have their nitrogen-fixing powers greatly increased when arsenic is applied to them. At least some soils high in organic matter fix as much nitrogen in the presence of arsenic and in the absence of mannite as they do in the presence of mannite and in the absence of arsenic. The stimulation is greatest when the water-soluble arsenic content of the soil is about 10 parts per million.

One type of *Azotobacter* has been isolated which is stimulated by arsenic, and in this case the stimulation is due to the organism utilizing more economically in the presence of arsenic its source of carbon than it does in the ab-



sence of arsenic. The arsenic compounds do not act as a source of energy to the organisms. The main part of the stimulation noted in the soil with its mixed flora is undoubtedly due to the arsenic inhibiting injurious species.

Arsenic cannot replace phosphorus in the vital process of the nitrogen-fixing organisms, but it may in some manner liberate the phosphorus from its insoluble compounds. This may be either a direct or an indirect action. Arsenic stimulates the cellulose ferments, which, in turn, react upon the activity of the nitrogen-fixing organisms. The nitrogen-fixing powers of soil extract, of filtered soil extract, and of soil dried for some time are only slightly stimulated by arsenic, showing that arsenic acts mainly by the removal of a thermolabile body which occurs in the soil.

In the experiments where lead has been applied to a soil it has been associated with arsenic, but the evidence that it stimulates nitrogen-fixing organisms when applied in small quantities is conclusive. The point at which it would become toxic is, however, unknown. Copper, on the other hand, is toxic even in the lowest concentration which has been so far tested.

#### VOLATILE ANTISEPTICS

Ether, carbon bisulfid, and other volatile (46) antiseptics usually (95, 98) increase nitrogen-fixation when added to the soil in small quantities. It is certain that they stimulate *Azotobacter* in pure cultures, but not to so great a degree as in mixed cultures. *A. chroococcum* is fairly resistant to carbon bisulfid (135), as it is killed in 24 hours in a solution containing 1.7 parts in 1,000 at 20°C., but it survives for 48 hours in moist soils which have been impregnated with the fumes of carbon bisulfide. Various theories have been advanced to account for these phenomena. These have been carefully analyzed by Kopeloff (103) and co-workers and will not be considered here in detail. Suffice it to state that the evidence points strongly to the conclusion that there are a number of factors at work, chief among which are the following:

(a) In small quantities the compounds directly stimulate the protoplasm of the organism and thus increase their physiological efficiency.

(b) Antiseptics simplify the bacterial flora of the soil, and the nitrogen-fixing organisms, being more resistant to the compounds than are some other organisms, survive and later multiply unhindered by other forms.

(c) The compounds may stimulate other organisms or classes of organisms which render the carbonaceous material of the soil more available to the nitrogen fixers, or possibly remove products which are injurious to them.

(d) The compounds may render more plant-food available in the soil. This may either be a direct interchange between the compounds and those of the soil or a dissolving of various substances surrounding the essential constituent, in either case liberating more available food for the *Azotobacter*.

It is interesting in this connection to note the explanation which Kruger and Heinze (109) make for the action of some forms of green manure on bac-

terial activity. It had long been known that mustard, when plowed under on nitrogen-poor soil, increased the next crop grown on that soil. It would appear from some facts already known that green mustard substances in the soil retard the formation of acid-forming species (72), thereby greatly simplifying the bacterial flora. These compounds seem to act upon the bacterial flora in a manner similar to that of a carbon bisulfid. These workers find theoretical support for this belief in the fact that allyl mustard oil,  $C_3H_5 - N = C = S$ , a constituent of the mustard plant, may be regarded as a derivative of carbon bisulfide.

#### ORGANIC SOIL CONSTITUENTS

Reed (164) found urea, glyocol, formamide, and allantoin active in depressing nitrogen-fixation. This he attributes to the compounds furnishing to the *Azotobacter* an available source of combined nitrogen and not to the direct toxic action of the compound. But Walton (201) found that the addition of urea, peptone, acetamid, asparagin, and casein to culture media had only a slight influence on the fixation of nitrogen by *Azotobacter*.

Caffeine, alloxan, betaine, trimethylamine, legumin, cinnamic acid, aspartic acid, asparagine, hippuric acid, creatin, creatinine, xanthine, and hypoxanthine, are all toxic to *Azotobacter* even in small quantities. Only the first two have been tested in concentrations dilute enough to stimulate, which is remarkable, as many of these compounds stimulate the higher plants and some can be utilized directly by the plant.

Esculin, vanillin, daphnetin, coumarin, pyrocatechin, heliotropin, arbutin, resorcin, pyrogallol, phloroglucine, hydroquinone, salicylic aldehyde, oxalic acid, quinic acid, dihydrostearic acid, rhamnose and borneol, on the other hand, do not stimulate in any concentration. Nor are they toxic until fairly large quantities have been added. In this regard the nitrogen-fixing organisms appear to differ greatly from the nitrifying bacteria and higher plants. The resistance of the nitrogen-fixers to various chemicals has likewise been called to our attention by Lipman (118) in his study of the influence of alkalies on nitrogen-fixation.

#### INFLUENCE OF COLLOIDS

It was recognized early in the study of nitrogen-fixation that when sterilized soil is added to a nutritive medium it greatly increased the quantity of nitrogen fixed. This condition is due to several factors and is partly explained by Krzemieniewski's (111) results wherein he found that nitrogen-fixation is decidedly increased by the addition of soil humus, either as free humic acid or as salts of potassium, sodium or calcium. Kaserer (88) maintains that this is due to the inorganic nutrients, especially to aluminum and silicic acid supplied to the microorganisms through the humus. This is probably true

in part, for the fixation varies with the humus derived from different sources. Moreover, artificial humus, prepared by boiling sugar with acids, fails to stimulate.

That much of the beneficial effect is due to the constituents in the humus appears likely from the results obtained by Sohngen (178) who found that colloidal iron oxide, aluminum oxide, and silicon oxide all greatly stimulated the nitrogen-fixing powers of *Azotobacter chroococcum*. This he attributed to the absorption of oxygen and nitrogen by the colloid, which he maintains would make them more readily available to the organism. The boiling of natural humus with hydrochloric acid would either remove the foreign material or change it from the colloidal form, and thus, as has been found to be the case, render it inert. Löhnis and Green (130) take exception to this explanation, for they found no absorptive action exerted by humus on either the nitrogen

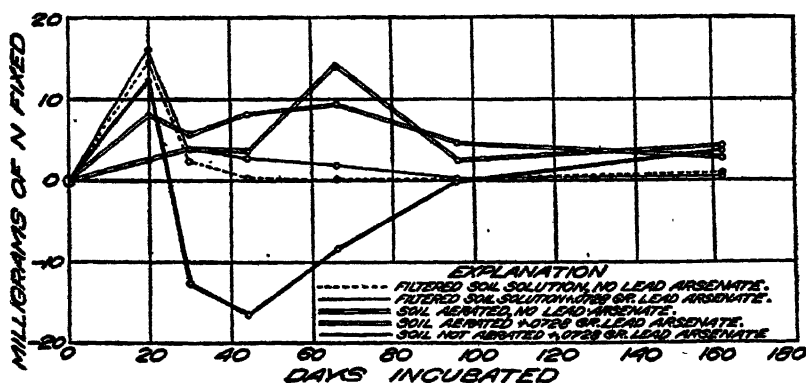


FIG. 1. GRAPH SHOWING THE EFFECT OF AERATION ON THE NITROGEN-FIXING ACTIVITY OF SOIL CONTAINING COMPOUNDS OF ARSENIC

or the oxygen. Furthermore, Rosing (169) found that he could stimulate just as effectively with iron as with humic acids. But much larger quantities of colloidal iron are required when it is used singly than when used in conjunction with an organic colloid (155). The extent of the stimulation resulting varies with the form in which the iron is applied and is most effective in the form of the hydroxide and in the presence of cane sugar (166). In this case it is probably the saccharate which is the active substance. Hence, the contradictory results reported may be due to the different mineral constituents of the humus.

These facts make it certain that colloids of the metals act as stimulants to nitrogen-fixing bacteria, as does also crude humus (141). Carefully purified humates do not possess this property, but it is possessed by the aqueous extract, the alcoholic extract, and the phosphotungstic fraction of the aqueous extract from "bacterized" peat. Whether this influence is due to a catalytic

effect, as suggested by Sohngen, or whether the substance furnished a direct source of nutritive material is not clear at the present time.

Moreover, the colloid may act as a protection to the organism against poison (56); for, when 10 parts per million of soluble arsenic is maintained in a soil, it acts as a stimulant to *Azotobacter*. If, however, this proportion is added to the Ashby nutritive solution it stops all nitrogen-fixation. This is due in part to the absorption of the arsenic by the soil. This absorption would have to be attributed largely to the silica compounds, for the nitrogen-fixing organisms are stimulated by arsenic in quartz free from organic colloids. This could readily be due to the arsenic becoming concentrated at the surface layers of the silica, leaving the inner part of the water film comparatively free from arsenic, in which part of the water film the microorganisms multiply and carry on their metabolic processes. This being the case, one should and probably could find a water solution weak enough to stimulate bacteria. A great difference, however, between the solution and the sand-culture method is the greater aeration in the sand. That the aeration of a culture medium does play an important part in determining the activity of the nitrogen-fixing powers of a soil is strikingly brought out in figure 1.

#### SOURCES OF ENERGY FOR THE AZOTOBACTER

The nitrogen-fixing organisms differ widely from other plants in their energy requirements. This is due to the fact that they are carrying on endothermic reactions in which nitrogen is concerned. This necessitates a greater supply of energy than is required by other bacteria. They are similar to most other bacteria in that this energy must be supplied by an organic compound, preferably one of the carbohydrates.

Berthelot (12) in his early work maintained that the gains in nitrogen noted in some soils were due to the action of biological agents on the humus of the soil. This was followed by the observation by others (75, 192, 84) that when forest leaves are allowed to decompose in soil there is an increase in its nitrogen content. Koch (98) in 1907 increased nitrogen-fixation by the addition to soil of dextrose, cane sugar or starch, but there was practically no increase when straw, filter paper or buckwheat was applied. Yet Stoklasa (187) showed that the decomposition products of these substances acted as a valuable source of energy to the *Azotobacter*, and Stranak (191) considered that the pentosans of the soil are of the greatest importance in the assimilation of nitrogen by soil bacteria.

A fair idea of the great variety and relative efficiency of substances which may serve as a source of energy to the azofiers may be obtained from the work of Löhnis and Pillai (132). They inoculated a nutritive solution with 10 gm. of soil and after 10 days determined the gain in nitrogen.

SUBSTANCE ADDED	NITROGEN FIXED AFTER 10 DAYS	SUBSTANCE ADDED	NITROGEN FIXED AFTER 10 DAYS
	<i>mgm.</i>		<i>mgm.</i>
Mannite.....	9.40	Starch.....	3.36
Xylose.....	9.54	Sodium tartrate.....	2.82
Lactose.....	9.12	Glycerine.....	1.68
Laevulose.....	8.52	Sodium succinate.....	2.96
Inulin.....	7.72	Calcium lactate.....	2.49
Galactose.....	7.86	Sodium citrate.....	1.42
Maltose.....	7.44	Sodium propionate.....	1.10
Arabinose.....	7.62	Potassium oxalate.....	0.12
Dextrin.....	7.18	Calcium butyrate.....	0.02
Sucrose.....	8.60	Humus.....	-0.96
Dextrose.....	4.62		

Other workers have noted larger gains of nitrogen than those noted by Löhnis and Pillai, but they can readily be attributed to (a) the time of incubation (81)—in this case, 10 days being far too short for the complete utilization of the carbonaceous substance applied; (b) the species of nitrogen-fixers which are bringing about the change; and (c) whether pure or mixed cultures are used. The order of effectiveness noted above, however, is that recognized by most workers. Brown and Allison (23), however, do report results in which greater fixation was obtained with dextrose than with mannite. But in this case, calcium or sodium carbonate seems to be even more necessary than it is with the mannite (186). Moreover, some species utilize one carbohydrate most effectively and another species a different one. To this list may be added malate, gum tragacanth, ethylene glycol, methyl, ethyl, and propyl alcohols, lactic, malic, succinic and glycollic acids. Fatty acids are readily utilized, the amount of nitrogen fixed being greater with the increased molecular weight, from 1.47 mgm. with formic acid, to 6.08 mgm. with butyric acid (140). Most of the naturally-occurring glucosides and many benzene derivatives are unsuitable as sources of energy for *Azotobacter*. Molasses, which should serve as a useful source of energy (95), often results in a loss of nitrogen when applied to the soil. This may be due to the time of applying, concerning which Peck (150) maintains that molasses applied to a land lying fallow at an interval of several weeks before planting of the crop may produce beneficial results by increasing nitrogen-fixation.

Beijerinck early recognized that certain decomposition products of cellulose can also serve as sources of energy for *Azotobacter*, and Pringsheim (157) found that *Clostridium americanum* does not fix atmospheric nitrogen in sterilized cellulose unless other carbohydrates like dextrose, lactose, mannitol, or sucrose are present. However, in the presence of cellulose, *Clostridium* will fix nitrogen and this more efficiently than it will in the regular carbohydrate medium. The same holds for agar (161). Just how completely cellu-

lose must be broken down before it can be utilized by *Azotobacter* is not definitely known, but it is known that *Azotobacter* cannot utilize cellobiose except when grown in conjunction with *Aspergillus niger*. It is, therefore, certain that the products which are utilized by the *Azotobacter* are comparatively simple.

Cellulose when applied to the soil may serve as a valuable source of energy, provided sufficient time is allowed for its decomposition (17, 97, 158). The cellulose ferment is probably the most efficient organism in the soil in bringing about this decomposition (136). But the number of soil fungi which possess this power are numerous (202).

Hoppe Zeyler (83) thinks that cellulose is decomposed according to the following formula: (a) the hydration of the cellulose with the formation of hexose,  $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$ ; (b) the destruction of the carbohydrate with the formation of equal quantities of carbon dioxide and methane,  $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4$ . None of the cellulose ferments studied by McBeth (137), however, yielded gaseous products on cellulose or sugar; hence the *Azotobacter* probably gets from the cellulose ferments, pentoses, hexoses, or slightly simpler products upon which they could readily fix nitrogen.

At times in fermenting straw and manure, the thermophilic anaerobic bacteria play a major part, in which case fatty acids probably make up the greater part of the end products (160).

It is claimed by Dvarak (37) that substances with low carbon and high oxygen content are usually the best sources of energy for *A. chroococcum*, which assimilated 5.73 mgm. of free nitrogen per 100 gm. of carbon in pine leaves as compared with 1237.9 mgm. per 100 gm. of carbon in red clover. He obtained for other substances the following results:

- 1456.5 mgm. of nitrogen per 100 gm. as glucose.
- 280.4 mgm. of nitrogen per 100 gm. as corn stalks.
- 596.8 mgm. of nitrogen per 100 gm. in stalks and root residues of corn.
- 325.4 mgm. of nitrogen per 100 gm. in wheat straw.

The carbon-nitrogen ratio (23) in compounds is no indication of their value to nitrogen-fixing organisms, for non-leguminous hays and straws are utilized just as effectively as are the legumes. Mockridge (140) found that the ratios of nitrogen fixed to the heat of combustion with the four lower fatty acids is almost constant. The same holds true with starch, dextrin and gum arabic, when allowance is made for experimental error, which is greater with these compounds than with the simpler compounds. This close relationship is not, however, general and no such graduated uniformity is observed with the series of monohydric alcohols.

The quantity of nitrogen fixed per gram of carbohydrate varies greatly with the species. Winogradski (210) found *Clostridium pasteurianum* to

assimilate 2 to 3 mgm. of nitrogen for each gram of sugar. But this, like other anaerobic organisms, is very wasteful of energy, leaving much of it in the butyric acid, acetic acid, and butyl alcohol formed. In the experiments of Bredeman with *B. amylobacter* and of Pringsheim with *Clostridium americanum*, the amounts fixed were at times much larger. Much greater fixations have been reported with *Azotobacter*, and Lipman has obtained as high as 15 to 20 mgm. of nitrogen per gram of mannite assimilated by *A. vinelandii*. This quantity is considerably greater than that fixed by any of the other members of the group.

Koch and Seydel (100) claim that the usual method of estimating the nitrogen-fixing powers of *Azotobacter* is erroneous, as it does not represent accurately the intensity of the process. In a series of experiments made by them, the amounts of nitrogen fixed per gram of dextrose used were 53, 70 to 80, 20 to 30, and 5 to 8 mgm. on the first, second, third, seventh, and eighth days, respectively.

Krainskii (107) considers that there should be sufficient organic matter in the soil to permit that for 1 part of nitrogen formed there will be 90 parts of carbon for the use of the organism. The organisms, however, utilize the carbohydrates more economically when only small quantities are present (81). Walton (201) finds with Ind an soil that highest fixation is obtained per gram of mannite when 10 gm. are used in 1 litre of nutritive solution. Young, vigorously-growing cultures usually fix more nitrogen than older ones (52). The nitrogen fixed is greatest in the first stages of the growth of the organisms, as is seen from figure 2 from the work of Omelianski (148).

The efficiency of these organisms is, therefore, greatest when they are rapidly multiplying and it decreases as their metabolic products accumulate (139). Hoffman and Hammer (81) claim this to be due in impure cultures to a loss of nitrogen or free ammonia occasioned by the decomposition of the cells of *Azotobacter*. This explanation would hardly hold in the presence of pure cultures, unless we ascribe the breaking down to an autolytic ferment secreted by the *Azotobacter* cell. According to Koch and Seydels (100) this indicates that in the latter stages of fixation, when there occurs an accumulation of nitrogenous material in the medium, the organisms employ the carbohydrates for other purposes than for nitrogen-fixation. Under natural conditions in the soil this accumulation and concentration of nitrogenous material by the *Azotobacter* is not likely to occur; hence, they assume that the organism will continue fixing nitrogen at the high ratio noted in the early part of laboratory experiments.

The quantity of nitrogen fixed, however, is dependent upon factors other than the source of energy; e.g., Krzemeniewski (112) found in experiments with *A. chroococcum* that the addition of humates to the cultural solutions increased the nitrogen fixed from a maximum of 2.4 mgm. to 14.9 mgm. Moreover, Krainskii (106) found *Azotobacter* to utilize from 100 to 200 gm. of

sugar in the assimilation of 1 gm. of nitrogen when grown in solution, but when grown on sand it required only 11 to 30 gm. for the same fixation.

They utilize their energy more economically in the presence of a liberal supply of phosphorus than when the quantity of available phosphorus is limited (38). This accounts, in a measure, for the high fixation noted in most Utah soils.

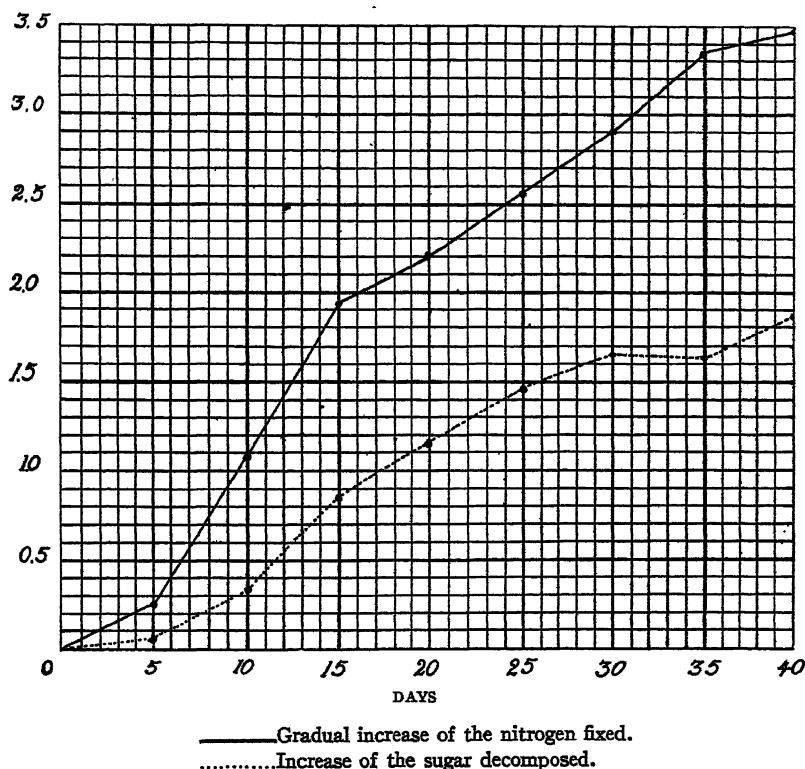


FIG. 2. GRAPH SHOWING THE FIXATION OF NITROGEN AND DECOMPOSITION OF SUGAR IN MIXED CULTURES OF *Azotobacter chroococcum* AND *Clostridium pasteurianum*

#### MANURE

It has been known for a long time that humus exerts a highly favorable effect on nitrogen-fixation. The great question, however, has been as to the manner of action. Humus, being such a complex variable substance, varies greatly in action, depending upon its source (111). Remy (165) considered that some of the products from humus are favorable sources of organic matter for *Azotobacter*. Definite and valuable information is furnished by the work of Löhnis and Green (130). They worked with mixed cultures of *A. chroococcum*, *A. beijernickii*, *A. vinelandii*, and *A. vitrium* in Beijernick's mannite solution with various forms of organic matter.



MATERIAL	NITROGEN FIXED IN 100 CC. OF SOLUTION AFTER 3 WEEKS
	<i>mgm.</i>
Fresh straw.....	10.0
Fresh stable manure.....	9.8
Fresh peat.....	9.3
Green manure.....	8.0
Beijerinck's mannite solution.....	5.6

After humification, these substances were even more readily assimilated and the nitrogen-fixation was greater than when the unhumified substance was used.

The same year Hanzawa (65) published results which show that stable manure even up to 3 per cent greatly stimulated the bacterial activities. Green-manure humus was found by him to be injurious. From this it is certain that humus can act as a source of energy and usually stimulates bacteria, but the extent is governed largely by its composition and by the quantity of available combined nitrogen which is being supplied with it to the organism. In addition to this, corn roots (37), corn stalks, oak leaves, lupine alfalfa, maple leaves, and pine needles may all serve as a useful source of energy to the nitrogen-fixing organisms. Apparently, the tissues from the non-legume give a greater gain than do those from the legumes (23). Fulmer (48) has recently confirmed these results.

The influence of stable manure upon the nitrogen-fixing powers of the soil under field conditions is seen from the following table in which the quantity of nitrogen fixed in the unmanured soil has been taken as 100 per cent (58).

TREATMENT	NITROGEN FIXED
	<i>per cent</i>
No manure.....	100
5 tons of manure per acre.....	103
10 tons of manure per acre.....	110
15 tons of manure per acre.....	105
20 tons of manure per acre.....	103
25 tons of manure per acre.....	101

These results indicate clearly that stable manure has a beneficial effect upon the nitrogen-fixing powers of the soil, but if used in large quantities the benefit is not so pronounced as if used in smaller quantities.

This decrease in nitrogen-fixation with increased additions of manure must be considered as due to its physical effect upon the soil, for Richards (167) found that *Azotobacter* grow and fix nitrogen in horse manure when it is well aerated and contains sufficient moisture and calcium carbonate. There is, too, a close connection between the diet and the effect. Horses fed on oats

gave feces which induced the greatest fixation; horses on grass next; cattle receiving oatmeal cake third; but the feces from cattle fed on grass proved unsuitable.

Manures often contain nitrogen-fixing organisms of considerable activity. Their activity appears to be greatest in fermenting manures mixed with straw which serves as a source of energy (194).

Although Fulmer and Fred (49) were unable to find *Azotobacter* in any of the samples of manure examined, they did obtain many nitrogen-fixing bacteria from it. One of these organisms, for which they suggested the name of *B. azophile*, is as efficient in fixing nitrogen as is *Azotobacter*. This would make it appear that manure may often carry to the soil nitrogen-fixing organisms.

#### METABOLISM OF AZOTOBACTER

Much time has been given to a study of the metabolism of *Azotobacter*, yet our knowledge of this subject is far from satisfactory. It is well known that the organisms oxidize the various carbohydrates and with the energy thus obtained build up complex nitrogen compounds. Berthelot (13) early recognized that the nitrogen so fixed is insoluble in water, thus indicating its protein nature. Lipman (123) found that there was a small but appreciable quantity of nitrogen in both young and old cultures of *A. vinelandii* not precipitated by lead acetate and a large proportion not precipitated by phosphotungstic or by tannic acid. Further work indicated that the substance was either amino-acids or comparatively simple peptids. He considered that one of early substances synthesized by these organisms is alanin. An analysis of the *Azotobacter* membrane gave the following results:

NITROGEN AS AMMONIA	BASIC NITROGEN	NON-BASIC NITROGEN	NITROGEN IN MgO PRECIPITATE	TOTAL PER CENT OF NITROGEN
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.98	2.76	6.39	0.42	10.45

This he finds corresponds remarkably close with that of legumin. Experiments with plants indicate that the nitrogen of the *Azotobacter* cells is not readily assimilated by plants (72).

Stoklasa found the *Azotobacter* cells to contain 10.2 per cent of total nitrogen and 8.6 per cent of ash. The ash contained from 58 to 62.35 per cent of phosphoric acid. The nitrogen and phosphorus were mainly in the form of nucleo-proteins and lecithin. The percentages of both nitrogen and phosphorus in the cell increase with age (81).

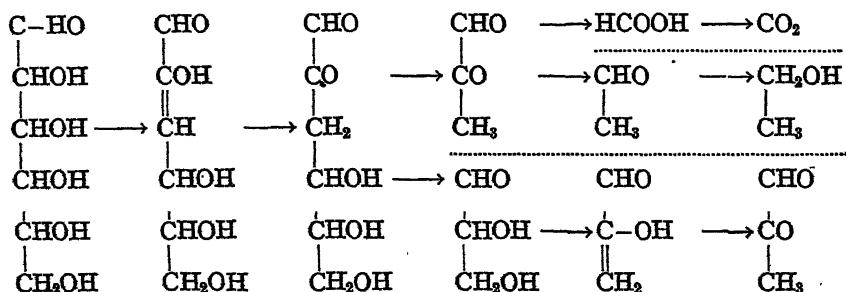
The most complete analyses of the *Azotobacter* cells, so far reported, show (147) them to contain, when grown on dextrin agar and rapidly dried at 30°C., 6.63 per cent of water, 4.12 per cent of ash, and 12.92 per cent of protein. The protein is similar to other plant proteins. It contains 10 per cent of

ammonia nitrogen, 26.5 per cent of diamino-nitrogen, and 60 per cent of monoamino-nitrogen. The quantity of lysine present is very high, but the histidine is present only in traces.

Krzemienwski (113) states that *Azotobacter* produces no hydrogen or other combustible gases in its metabolism, but according to Stoklasa (187) it does and in the presence of nitrates it produces ammonia and nitrites. Moler (142) claims that during its life, *A. chroococcum* separates no soluble compounds and it is only after death that it furnishes nitrogen to higher organisms. Nor are their bodies readily broken down by proteolytic enzymes. Both *A. agilis* and *A. vinelandii* separate a soluble nitrogen compound. The protein compounds so formed in soil are quickly broken down by other bacteria (42). Remy (165) considers the nitrogen fixed by *Azotobacter* in a readily available form for plant assimilation. Beijerinck found that 50 per cent of the total nitrogen in *Azotobacter* cells when supplied to the soil is nitrified in about seven weeks. None of the *Azotobacter* so far studied produce nitrates in the media (90).

Turning now to the breaking down of the carbohydrate, we find that the organisms produce ethyl alcohol (187); glycolic acid, acetic acid, butyric acid, lactic acid, carbon dioxide and hydrogen. The quantity and quality of the different products vary with the species and with the carbohydrate used (112, 132).

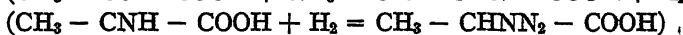
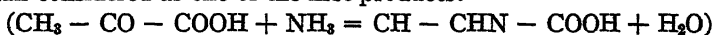
It is likely that many of the end-products have not yet been determined, for Stoklasa (187) starting with 15.9 gm. of dextrose recovered 7.9 as carbon dioxide, 0.3 as ethyl alcohol, 0.2 as formic acid, 0.7 as acetic acid, 0.2 as lactic acid, but could not trace the remaining 6.6 gm. The organisms are extremely active when growing under appropriate conditions, for 1 gm. weight of *Azotobacter* has evolved no less than 1.3 gm. of carbon dioxide in 24 hours (185). A great distinction between the *Azotobacter* and the other species is that the former decompose their sugar with carbon dioxide as the chief product, whereas the other species form large quantities of butyric acid. Some of these products may be accounted for as follows:



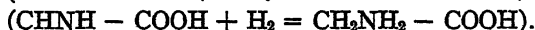
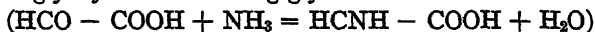
It is known that when sugars, such as glucose, levulose and mannose are acted upon by alkalis, there are produced a great many products, some of

which are formic, carbonic, oxalic, lactic, pyruvic, tartronic, malic, molonic, tartaric, rebonic, saccharic, and gluconic acids in addition to many other either more or less complex compounds. We can readily conceive that the *Azotobacter* bring about a somewhat similar reaction, the stages, however, being more nicely governed, because of enzymes. Many of the products would be oxidized to carbon dioxide and water with the liberation of energy necessary for the endothermic nitrogen reaction; others readily react with the formed nitrogen compound. We are completely in the dark as to what this first nitrogen compound is, but we know that the *Azotobacter* possess the power of changing nitrates or nitrites under appropriate conditions into ammonia. Up to date it has been impossible to detect nitrate formation; it is not impossible that nitrates are formed and utilized by intra-cellular enzymes. By using nitrates, nitrites or ammonia, we can offer a rough explanation of protein anabolism.

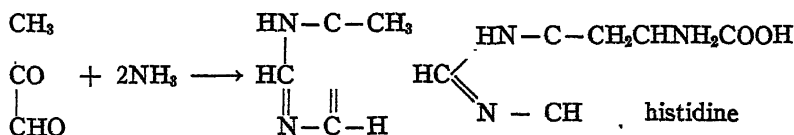
The endo-thermic reaction,  $2N + 2H_2O = NH_4NO_2$ , may take place and the ammonia thus formed may react with the decomposition products of the sugars—pyruvic acid, for instance—with the formation of alanine which Lipman considered as one of the first products:



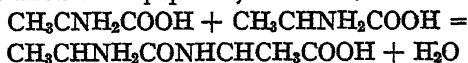
or with glyoxylic acid forming glycocoll:



By similar reactions other amino acids may be formed. Moreover, Windas and Knoop (208) have shown that methylimidazol may be produced from glucose and ammonia, presumably through the formation of pyruvic aldehyde and formaldehyde, which is nearly related to the amino acid, histidine:



The various amino acids may, through the intervention of proteinases, condense with the formation of dipeptides, thus:



By the continuation of this process and by condensing with phosphorus—and sulfur-bearing compounds, probably through the intervention of other enzymes, there may result the complex protein of the *Azotobacter* cell.

#### PIGMENT PRODUCTION BY AZOTOBACTER

Most species of *Azotobacter* produce pigments. These vary in color from brown to black of the *A. chroococcum*, to a yellow or orange of the *A. vine-*

*landii*. The pigmented film usually develops on the culture media in from 3 to 7 days (105). It is formed by *A. chroococcum* earlier and in more abundance where old brownish cultures are used as the inoculating material. It is produced and retained within the bacterial cell; it occurs in neither the capsule nor the medium (86). The pigment produced by *A. chroococcum* is most pronounced when a dextrin agar medium to which calcium carbonate is added is kept at a temperature of 30°C. under well-aerated conditions. According to Jones (86), it is produced only when there is a lack of suitable available nutrient material and when organisms in the pigment area have ceased to multiply. The color of the pigment is intensified if nitrates (171) are added to the medium in which the organism is growing. The non-pigmented strains apparently fix nitrogen just as readily as do those which have not lost the power of forming pigments.

The pigment from *Azotobacter chroococcum* is insoluble in water, alcohol, ether, chloroform, benzol, and carbon bisulfide (149). It dissolves in alkalies, undergoing decomposition with the formation of a dark brown solution. Sackett (171) maintains that the peculiar brownish color which is characteristic of certain "nitre spots" of some soils is due to the pigment produced by *Azotobacter*. Such soils are high in nitrates and alkalies which would dissolve the pigments from the body of the organism. But Omelianski and Sswewrowa (149) are of the opinion that, although in some cases the dark color of vegetable soil may be due in a measure to the action of these microorganisms, it would be a mistake to attribute it to this factor alone. Furthermore, it has recently been proved (182) that the brown color of the "nitre spots" is due to solvent and decomposing action of the nitrates on the colored organic compounds of the soil, for they may be produced at will in a rich greenhouse soil with an excess of sodium nitrate, and this too in soils which have been rendered sterile with a saturated solution of mercuric chloride.

#### MORPHOLOGY OF THE NITROGEN-FIXING ORGANISMS

Of the many different bacteria which have been isolated and proved to have the ability to assimilate free nitrogen, *Clostridium pasteurianum* may be taken as a type of the anaerobic and *Azotobacter chroococcum* as a type of the aerobic.

*Clostridium pasteurianum* (211) is a short thick rod, from 1.2 to 1.3  $\mu$  in diameter and 1.5 to 2  $\mu$  long, in the young cells; the older spore-bearing cells take on a spindle shape. The bacteria stain a violet brown with iodine. The spores when ripe are 1.6  $\mu$  long and 1.3  $\mu$  broad and often lie in a roughly triangular covering. The ripe spore escapes through the wall of the mother in a longitudinal direction. Their germination is polar.

*Azotobacter chroococcum* occurs ordinarily as diplococci or short rounded rods 1 to 2  $\mu$  thick and 1.5 to 3  $\mu$  long, and according to Prazmowski (154) the microorganism first presents itself in its vegetative stage as a bacterium in the

fruiting stage as a micrococcus, and possesses a nucleus which functions in the same way as that of higher animals. In the resting stage the nucleus assumes a globular form, having a strongly refractive nucleolus with clearly differentiated boundary layers. The individuality of the nucleus appear to be practically lost at times, because of its relation to the cytoplasm. The division of the nucleus marks the first stage of cell division. According to Bonazzi (15) the organism shows peculiar granulations apparently not related to reproduction. These take the basic dyes and are neither fats, glycogen, starch nor chromatin, but appear to be of a metachromatic nature and seem to have their genesis in the nucleus. Their disposition in the cells is not constant but changes in different individuals. Involution forms occur and cell division is preceded by a simple form of mitosis (138). Some, but not all, varieties have been observed to form spores (131). The volutin bodies within the organism increase in number and size when the organisms are grown on media rich in nitrates. Hills (77) suggests that they may have some relation to nitrogen-fixation, but his results appear to oppose this view; whereas the addition of nitrates to a media greatly increased the reproduction, it very materially decreased the physiological efficiency of the organism. It seems, therefore, more likely that they are reserve protein material.

Löhnis and Smith (133) have recently observed that *Azotobacter*, in common with many other bacteria, pass through a life cycle which is not less complicated than those of other microorganisms. Under certain conditions they pass over into an amorphous or "sympastic" stage, appearing under the microscope either as an unstainable or a readily stainable mass without any easily distinguishable organization, which, if not discarded as dead, later gives rise to new regenerative forms. They multiply not only by fission, but by the formation of gonidia.

#### METHODS

*Clostridium pasteurianum* grows readily in a vacuum on carrots. The organism also grows on sliced potatoes, but ordinarily is grown in an aqueous solution containing 1 gm.  $K_2PO_4$ , 0.5 gm.  $MgSO_4$ , 0.01 to 0.02 gm. NaCl,  $FeSO_4$ , and  $MnSO_4$ , and 1.0 gm.  $CaCO_3$ , and 10 to 15 gm. of a suitable carbohydrate in 1 litre of water. One method used by Winogradsky in isolating *B. Clostridium pasteurianum* was to add garden soil to a non-nitrogenous solution and to allow a stream of nitrogen gas to pass through the solutions, after which several successive transfers were made into similar media. The final culture, after *B. Clostridium pasteurianum* had formed spores, was heated to 80°C.

The organism ferments certain carbohydrates with the formation of butyric acid acetic acid, carbon dioxide, and water. When grown in nutritive solution devoid of combined nitrogen, it assimilates atmospheric nitrogen, although in pure cultures it is an anaerobe. In nature it occurs in connection with two other bacteria which do not possess the power of fixing nitrogen, but

the nitrogen requirements of which are small. When in conjunction with these organisms *Clostridium pasteurianum* has the ability of growing in the upper layers of soil and of assimilating free nitrogen.

*Azotobacter chroococcum* grows readily on solid or liquid media, one of the best being:

	<i>per cent</i>
Monopotassium phosphate neutralized to phenolphthalein by sodium hydroxide	0.02
Magnesium sulfate.....	0.02
Sodium chloride.....	0.02
Calcium sulfate.....	0.01
Ferric chloride (1 per cent solution) 2 drops per 100 cc.	
Mannite.....	1.00

The organism is readily isolated by seeding this medium with soil. When the characteristic membrane forms, it is transferred by dilution to a similar medium containing agar in which the characteristic brownish black colonies form readily.

On mannite agar the colonies first appear as milky white glistening drops, round and convex, which under a low magnification show a coarsely granular structure extending to the margin. The colonies rapidly increase in size, and after a week or more become brown at the center with concentric rings alternating dark and white to the circumference and darker streaks radiating from the center outward. In old cultures, where the agar has partly dried up, the cells are often united in sarcina-like packets; the cell walls are much swollen and the contents are aggregated to a small ball at the center. At the same time giant cells, both round and elongated and filled with oil drops, can be seen. Often too a number of involution forms are seen, drawn out with long threads and false septa (2). By successive dilutions and transfers, it may be obtained in pure culture, although at times considerable difficulty is experienced in freeing it from a small organism—*B. radiobacter*.

Several different methods have been used for studying its nitrogen-fixing powers:

- (a) Seeding into 100 cc. of the medium given above and after a certain time determining the nitrogen.
- (b) The use of the same medium, but the addition of sufficient sand for the formation of sand slopes on which the organism can grow.
- (c) The addition of a definite quantity of a carbohydrate to a soil and the incubation of this.

Each of these methods has its value. The first is much more readily handled in the final Kjeldahl determination, but the others give much higher results.

Freudenreich (47) found that when *Azotobacter* are grown upon gypsum, the gain in nitrogen is considerably in excess of that assimilated in the liquid media. Krainski (106) found *Azotobacter* to utilize from 100 to 200 gm. of sugar in the assimilation of 1 gm. of nitrogen when grown in solution, but

when grown on sand it required only 11 to 30 gm. for the same fixation. Many other workers have noted similar variation when grown in the soil. Where the organisms have been grown on gypsum or soil, we may attribute the stimulation to certain soluble constituents, yet this explanation scarcely seems plausible when considered in relation to sand cultures. Three strains of *Azotobacter* were grown in Ashby's mannite solution and sand (nearly pure silicon dioxide) to which Ashby's solution was added, with the following results:

	NITROGEN FIXED IN ASHBY'S SOLUTION	NITROGEN FIXED IN SAND
	mgm.	mgm.
<i>Azotobacter</i> A.....	6.86	22.61
<i>Azotobacter</i> B.....	5.00	12.60
<i>Azotobacter</i> C.....	6.44	16.80

Moreover, arsenic is very toxic in the solution, whereas when added to the soil or to pure quartz, in small quantities, it stimulates. Although the total quantities of nitrogen fixed under the two methods differ greatly, the relative efficiency of the organisms is about the same in both cases. In the testing of soils the same results are obtained, as may be seen from the following results, which is the average for several hundred determinations made on different soils by the two methods.

DEPTH OF SAMPLE	NITROGEN FIXED IN	
	100 gm. of soil + 1.5 gm. of mannite	100 cc. of Ashby's solution containing 1.5 gm. of mannite
	mgm.	mgm.
First foot.....	5.28	2.11
Second foot.....	2.42	0.77
Third foot.....	1.55	0.58

Although the greater aeration in the sand and soil culture probably plays a great part, there is little doubt that the colloids also assist.

#### RELATION OF AZOTOBACTER TO OTHER ORGANISMS

In the early study of nitrogen-fixation, the view was held that algæ growing on or near the surface of soil are able to fix nitrogen. Frank (44) in 1888 had observed such a growth on sand exposed to light and found that the soil showed a considerable increase in nitrogen. In 1892 Schloesing and Laurent (174) proved, both by determining the nitrogen fixed by a soil in a closed vessel and by observing the diminution of the nitrogen gas in the enclosed air, that a soil exposed to light gains in nitrogen if algæ are allowed to grow on the surface, and that the gain is confined to the upper few millimeters. They



did not, however, employ a pure soil or pure cultures of algæ. Kossowitsch (104), working with pure cultures of two green algæ, found no fixation, but observed a considerable increase of soil nitrogen when they were grown with soil bacteria. Later, Krüger and Schneidewind (110), employing pure cultures of many other chlorophyceæ, obtained no nitrogen-fixation. Hellriegel and Deherain had found a large increase in the nitrogen content of sand in pots when exposed to the light, which was always accompanied by a development of algæ. In the light of such results, the conclusion has been reached that algæ alone cannot assimilate free nitrogen, but only in concurrence with soil bacteria, the former producing carbohydrates which are used by the latter as a source of energy for the nitrogen-fixation. Heinze (73) actually observed rapid fixation of nitrogen when cultures of algæ were inoculated with *Azotobacter* or other nitrogen-fixing organisms. Stoklasa (138) found that *Azotobacter* are especially abundant in soils having a vigorous growth of blue-green algæ. *Azotobacter* are often absent from virgin soils, but are always found in such soils when there is a vigorous growth of algæ (191). Bottomley (18) claims that both *Azotobacter* and *Pseudomonas* live in true symbiosis with cycas. It, therefore, appears certain that the nitrogen-fixing powers of *Azotobacter* are greatly enhanced when growing with algæ, but the exact rôle played by each is yet to be explained. This offers a rich and inviting field for research.

Nor is it alone in combination with algæ that these organisms may grow and thus be benefited. Beijerinck and Van Delden (9) early recognized that an apparent symbiosis exists between *Azotobacter* and other bacteria, and that the nitrogen fixed is considerably greater in the mixed than in the pure cultures. This symbiosis, though in many cases beneficial to *Azotobacter*, is not essential for nitrogen-fixation (123). Radiobacter, with which the *Azotobacter* are usually associated, have only slight nitrogen-fixing powers (187), yet they increase the nitrogen-fixing powers of *Azotobacter* (112). The carbohydrates disappear more rapidly from mixed than from pure cultures and with a greater fixation per gram of carbohydrate utilized (17). There is also a greater fixation when two strains of *Azotobacter* are grown in conjunction with each other. This is especially marked in an aqueous solution of mannite (65). Results have been reported (16) where *Azotobacter* fixed twice as much nitrogen in the presence of *Pseudomonas* as when grown alone.

The manner in which this mutual benefit is exerted is not clear. In some cases it may be due to the associated organism rendering more available the carbonaceous material.

Omelianski and Salunskov (148) offer the following explanation concerning the association of aerobic and anaerobic nitrogen-fixers:

The synergetic activity of nitrogen-fixing and accompanying microbes, is both in laboratory experiments and under natural conditions (cultivable stratum of the soil) of a different character according to the properties of the species taking part in the process and their environment; in both cases the function of the satellite organism seems to consist in

fixing the oxygen of the air and creating the anaerobic environment for *Clostridium pasteurianum*. The species added to the cultures of nitrogen-fixing microbes sometimes supply the compounds of carbon needed for the process of fixing nitrogen as energetic substance. In the case of the combination: *Azotobacter* + *Clostridium pasteurianum*, the function of the former is not confined to fixing the oxygen of the air only, and consequently to creating an anaerobic environment for the *Clostridium*. But this combination is also useful inasmuch as it destroys the injurious products of disassimilation created by the second (chiefly butyric acid) and maintains the action of the environment. (*Azotobacter* is alkaligenic and the *Clostridium* acidogenic.)

The satellite species may also unfavorably affect the nitrogen-fixing microbe, either through products of assimilation or by consumption of the carbon compounds needed by this microbe for nitrogen-fixing. The energetic fixation of oxygen by the satellite aerobic species creates conditions favorable to the development of *Clostridium pasteurianum*, but at the same time hinders the growth of the *Azotobacter*, which is necessarily aerobic.

The form endowed with the maximum vitality and at the same time the most common form in which combination of the nitrogen-fixing organisms takes place in the upper soil strata is that of symbiosis between the aerobic and anaerobic nitrogen fixers, principally between *Azotobacter* and *Clostridium pasteurianum*. In spite of the opposite properties of the two species, their synergetic activity in the upper strata of the soil results in a harmonious mutual development producing the maximum economy in consumption of energetic substances.

So far, little has been done to determine the relationship of *Azotobacter* to the higher plants, but it is interesting to note that Beijerinck (8) has observed a distinct relationship between the distribution of the organism and leguminous plants. Fischer (42) suggests that some nitrogen-fixing bacteria presumably exist first as saprophytes, then as exoparasites in loose combination with green plants, then as endoparasites. Finally they develop the true symbiosis of root nodule bacilli. Hopkins (82) has questioned whether there may not be a relationship between the legume bacteria and *Azotobacter*.

#### THE INFLUENCE OF WATER

*Azotobacter* are very resistant to drying; they may be dried for a considerable time in a desiccator over sulfuric acid. Pure cultures are just as resistant to drying as are mixed cultures (89). This would vary some with the media in which the bacteria are dried, for the survival of non-spore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a solid is not directly proportional to its grain size and hygroscopic moisture. Giltner and Langworth (53) found that bacteria resisted desiccation longer in a rich clay loam than in sand. Furthermore, if bacteria are suspended in the extract from a rich clay loam before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in a physiological salt solution. Because of this, they consider that soils contain substances which have a protective influence upon bacteria subject to desiccation.

Lipman and Burgess (117) have found that many soils manifest a vigorous nitrogen-fixing power even after being air-dried and kept in stoppered museum

bottles for periods varying from 5 to 20 years. In some cases the fixation was equally as high as in freshly-collected samples. The organisms from such soils are more easily attenuated than are other organisms which have not been so dried (207). The tendency is for soils gradually to decline in nitrogen-fixing power on drying. This may manifest itself as early as the second week.

During the periods of drying, the organisms are inactive, as they require moisture for growth and reproduction. For maximum nitrogen-fixation a definite moisture content is needed. Warmbold (203) found the optimum moisture content to be 20 per cent. When it was below 10 per cent there was no nitrogen fixed, and in some cases there was a decided loss of nitrogen. Krainski (105) allowed soil with varying moisture content to stand for some time and then inoculated it into mannite solutions and obtained maximum fixation in the soils containing fairly small quantities of water. Later, however, he decided that soil should be damp—but not wet—and well aerated for maximum nitrogen-fixation. The water requirements vary with different soils. As a general rule, the higher the humus content of the soil, the more water will be required for optimum nitrogen-fixation (108). The quantity of water present may, however, become so great that it may kill all *Azotobacter* in addition to stopping nitrogen-fixation (42).

An insufficient supply of moisture checks both nitrification and nitrogen-fixation (34). This occurs in some soils when the water content has been reduced to 16.5 per cent. This again varies with the soil, for Schloesing (173) found bacterial activity less in fine-grained soils than in lighter, coarse-grained soils. A difference in moisture content of 1 per cent, according to Defert and Bollinger (32), is sufficient to produce a marked change in the oxidation going on in the soil.

The moisture requirement of the nitrogen-fixing bacteria, according to Lipman and Sharp (119), is more nearly that of the ammonifying than of nitrifying organisms. In a sandy loam it was found to vary between 20 and 24 per cent of moisture in the soil. At the higher percentages of moisture up to 24 per cent the anaerobic nitrogen-fixers are most active, but the action of the aerobes is slightly depressed. Thus, in many soils two maxima of nitrogen-fixation occur, depending upon whether the conditions are favorable for the anaerobic or aerobic organisms.

Traaen's results (195) differ from Lipman's in showing only the one maximum, as is seen from the following, which gives the milligrams of nitrogen fixed in 100 gm. of soil.

TEMPERATURE	5 PER CENT H <sub>2</sub> O	10 PER CENT H <sub>2</sub> O	17.5 PER CENT H <sub>2</sub> O	25 PER CENT H <sub>2</sub> O	30 PER CENT H <sub>2</sub> O
13°C.	0.1	1.5	11.2	13.4	5.4
25°C.	1.9	1.9	13.2	16.6	15.5

He used a loam soil with a maximum water capacity of 27.4 per cent. It is quite evident from his statement that anaerobic organisms played a prominent part in the fixation at the higher moisture contents.

Since the carbohydrates disappeared much more rapidly in the soils containing the greater quantities of water, it is quite possible that greater quantities of nitrogen per gram of carbohydrate consumed are fixed where the smaller quantities of water are applied. This, together with the different methods used by the several investigators, would explain the apparent discrepancy in their results.

In a series of pot experiments in which a calcareous loam receiving various amounts of water was used, the author (58) found the moisture content for maximum nitrogen-fixation to lie between 15 and 22 per cent. These results also bring out the two maxima which were first noted by Lipman. These soils were kept at the various moisture contents for four months. All were then incubated at 28°C. for 21 days with a moisture content of 20 per cent.

TREATMENT	NITROGEN FIXED
<i>per cent</i>	<i>per cent</i>
12.5	100
15.0	108
17.5	102
20.5	104
22.5	108

In this soil the optimum for the aerobes would appear to be at 17.5 per cent and that for the anaerobes 22.5 per cent or higher.

When too large a quantity of water is applied there is a tendency to depress the total nitrogen fixed, as is illustrated by the following results in which various quantities of water were applied to a soil throughout the year under field conditions (59).

37.5 inches of water applied during summer; 1.4 mgm. of nitrogen fixed in 100 gm. of soil.  
 25.0 inches of water applied during summer; 2.1 mgm. of nitrogen fixed in 100 gm. of soil.  
 15.0 inches of water applied during summer; 8.5 mgm. of nitrogen fixed in 100 gm. of soil.  
 No water applied during summer; 3.5 mgm. of nitrogen fixed in 100 gm. of soil.

The maximum for anaerobic conditions does not appear in these results probably because the soil did not become filled with water and because under field conditions the water rapidly drains away or is evaporated.

#### TEMPERATURE

Berthelot (13) early recognized that the biological gain of nitrogen in soils is dependent upon a suitable temperature. He found nitrogen-fixation to occur best at summer temperatures between 50° and 104°F. The process was

immediately stopped on heating to 230° F. Later Thiele (193) maintained that although *Azotobacter* possess the ability to fix small quantities of nitrogen under laboratory conditions, the temperature would be unfavorable under field conditions. Heinze (72), however, found that although the nitrogen-assimilating organisms are most active at a temperature between 20°C. and 30°C., they nevertheless fix appreciable quantities at temperatures as low as 8 to 10°C. Still more recent work (111, 134) has shown the optimum temperature to be 28°C., and the limits of activity of *Azotobacter chroococcum* to lie between 9°C. and 33°C. The actual quantitative variation in nitrogen fixed is seen from results reported by Löhnis (128). He inoculated 100 cc. of a 1-per cent mannite soil extract with 10 gm. of soil and obtained the following fixation at the various temperatures:

10° to 12°C.	20° to 22°C.	30° to 32°C.
3.15 mgm. nitrogen	4.55 mgm. nitrogen	4.27 mgm. nitrogen

Better fixation at a lower temperature is noted when the soil is incubated and the gain in nitrogen determined directly. Koch (95) obtained fixations of 3 mgm., 11 mgm., and 15.5 mgm. of nitrogen in 100 gm. of soil when incubated with a carbohydrate at 7°C., 15°C., and 24°C., respectively. Traaen (195), using a loam soil with a maximum water-holding capacity of 27.4 per cent, obtained nearly as great a fixation at 13°C. as at 25°C. when the optimum moisture content was maintained. This is seen from the following:

TEMPERATURE	NITROGEN FIXED IN 100 GM. OF SOIL				
	5 per cent H <sub>2</sub> O	10 per cent H <sub>2</sub> O	17.5 per cent H <sub>2</sub> O	25 per cent H <sub>2</sub> O	30 per cent H <sub>2</sub> O
	mgm.	mgm.	mgm.	mgm.	mgm.
13°C.	0.1	1.5	11.2	13.4	5.4
25°C.	1.9	1.9	13.2	16.6	15.5

A temperature, favorable even though not ideal for nitrogen-fixation, would occur in soils under natural conditions. The temperature of soil in Utah during the months of September averaged 14°C., with a minimum of 10°C. and a maximum of 17°C. During June, July and August the mean temperatures would be much greater.

The mean daily temperatures of the soil for Bismarck, North Dakota; Key West, Florida; and New Brunswick, New Jersey; for the months of June, July, August and September were 18°C., 28°C., and 24.5°C., respectively. From this it is evident that during a considerable period of each year an arable soil has a temperature high enough for moderately rapid nitrogen-fixation.

Although it is generally maintained that there is no nitrogen-fixation in soils during the winter months, cold or even freezing does not injure the or-

ganism; for the cooling of a soil, even to the freezing point, increases its nitrogen-fixing powers (24). This is probably due to the suppression of competing species and to the establishment of a new flora. The same is true when the soil is heated, as may be seen from the results given below (56).

TEMPERATURE	NITROGEN FIXED
deg. C.	mgm.
Normal	5.11
50	9.00
55	14.14
60	16.38
65	14.42
70	13.02
75	11.34
80	12.66
85	10.36

This soil had been autoclaved and then inoculated with a soil extract which had been heated to the temperature indicated. The stimulation could not, therefore, have been due to the heat rendering more of the plant-food in the soil available. The results indicate that many of the organisms which take part in nitrogen-fixation are highly resistant to heat. It is significant that the greatest stimulation is exerted in a soil which had been inoculated with solutions heated just above the temperature which Cunningham and Löhnis (31) found to be the thermal death-point of soil protozoa.

#### LIGHT AND OTHER RAYS

As a class, bacteria are sensitive to light, but the extent to which they can withstand it varies, among other things, with the conditions of exposure and the specific organism. Unfortunately, we have but fragmentary information concerning the effect of light upon azofiers, but what we do know would lead us to believe they are more resistant than many microorganisms—probably more so than the many other soil bacteria. Berthelot (13) recognized that nitrogen-fixation in the soil occurred both in daylight and in darkness, though more freely in the light. Jones (86) found many *Azotobacter* to be alive in a small Petri dish of dried soil that had stood in the laboratory in front of a south window for two years. They can withstand the direct action of the violet and of the longer ultra-violet rays for five minutes (190), but are killed in much less time by the shorter ultra-violet rays. They are more resistant even to these than are many other species.

The fixation of elementary nitrogen by *A. chroococcum* is distinctly increased when the air is activated by pitchblende. Somewhat better results are obtained with weak than with stronger radio-active intensity.

## AERATION

Under field conditions there is a mixed flora consisting of the anaerobic and aerobic nitrogen-fixing microorganisms. A soil condition which would be ideal for one species might be unfavorable for the other. It has already been pointed out that there are two maxima of nitrogen-fixation in soils, depending upon the moisture content. This is illustrated in figure 1.

Although it is usually conceded that nitrogen-fixation is most rapid when soils are well aerated, this may not always be the case. Concerning this Murray (145) reports the following results:

KIND OF SOIL	NITROGEN FIXED	
	Aerobic conditions	Anaerobic condition
	mgm.	mgm.
Greenhouse soil.....	0.84	8.50
Loam soil.....	3.08	5.29
Clay soil.....	0.84	4.69

This condition must be attributed to a great difference in the physiological efficiency of the two groups found in these particular soils and not to a lack of aerobic nitrogen-fixing organisms, for more than ten times the number of organisms developed on nitrogen-poor media from these soils under aerobic as under anaerobic conditions.

## SEASON

Berthelot (13) was unable to show any gains in nitrogen of his soils during the winter, but Koch (94) found a considerable increase during the season in soils which were kept in a heap and shoveled over from time to time. Löhnis (127) observed that *Azotobacter* membranes are more readily obtained in winter than in summer. He later found that the nitrogen-fixing power of soil varies from month to month throughout the year, there being two maxima—one in spring and another in autumn (128). The extent of the variation noted may be seen from the following:

1903-1904.....	March	May	July	September
	100*	121	50	100
1907.....	April	May-June	July-August	October-November
	100*	133	69	122

\* The relative numbers are based on the spring months as 100.

Green (60) found nitrogen-fixation in 1 per cent mannite solution to be low during August, September and April. In other months he noted a fairly constant fixation of about 10 mgm. of nitrogen per gram of mannite. He also noted a marked yearly variation in the nitrogen fixed during July and August.

Walton (201) found nitrogen-fixation lowest in Indian soil between October and January and highest between June and September. This corresponds with moisture and temperature changes. Peterson (153) has found that although the nitrogen-fixation of Utah soils is highest from June to September, the number of types of *Azotobacter* occurring in the soil was greatest in May. Moll (143) goes so far as to maintain from his work that the season of the year is the principal factor in determining the biochemical transformations in soils. This would appear to be especially true as regards nitrogen-fixation.

## CROP

Heinze (72, 74) called attention to the fact that the fallowing of the soil increased its nitrogen-fixing power. This could be due to better aeration, moisture, temperature, etc., and not to any depressing influence exerted directly by the plant. Most experiments which consider plant and bacterial activity could be interpreted in this light. Hiltner (78) maintains that the free nitrogen-fixing bacteria are stimulated in their activities by the growing plant roots. There may be considerable truth in this, for here the higher plants are rapidly removing from the solution the soluble nitrogen compounds. In this case, the nitrogen-fixing organisms would be forced either to compete with the higher plant for the soil nitrogen or else to make use of their ability to live upon the atmospheric nitrogen. It is certain that different cultural methods vary sufficiently with crops to influence profoundly a soil's nitrogen-assimilating properties, for the *Azotobacter* occur more widely distributed in cultivated than in virgin soil (74). The analyses of hundreds of samples of cultivated and virgin soils in Utah (55) have in nearly every case shown the virgin soil to have a low nitrogen-fixing power as compared with the cultivated soil. This was the case even where the soil was incubated without carbohydrates and the nitrogen determined directly. The average results for many determinations were as follows:

	<i>mgm. of nitrogen fixed</i>
Virgin soil.....	6.99
Cultivated .....	14.28
Wheat.....	11.83
Alfalfa.....	12.24
Fallow.....	22.81

Because the fallow soil had received considerable manure, the results are undoubtedly high. It would, however, be possible to fallow or crop soils so continuously that extremely small quantities of plant residues would be returned to the soil, under which conditions there might be a decrease in nitrogen-fixation. The conditions of moisture and aeration are much more nearly ideal in a fallow soil than in a cropped soil. It is just possible that the high fixation noted where wheat is grown continuously may be due to the method



in vogue in the arid districts of leaving the greater part of the straw on the soil. This would act as a readily assimilable carbonaceous material for the *Azotobacter*. Welbel and Winkler (205) have found that fallowing not only increases the assimilable nitrogen but also the available phosphorus of the soil, a liberal supply of which causes the *Azotobacter* to utilize its energy more economically. That the increased nitrogen-fixation noted when soils are cultivated is not confined to the arid soils, is seen from the recent work of Reed and Williams (163). Brown's work (22) indicates that crop rotation increases the nitrogen-fixing powers of a soil.

#### CLIMATE

It has been maintained for a long time that there is a close correlation between the chemical, physical, and biological transformations going on in a soil and the climatic conditions, but there was nothing definite on this subject until the highly interesting work of Lipman and Waynick (120) appeared. They found a definite relationship between climate and the nitrogen-fixing powers of a soil. Removal of California soil to Kansas increased the vigor of the *Azotobacter* flora and especially that of *A. chroococcum*. It increased the nitrogen-fixation by 50 per cent over that attained by the same soil in California. Similar results were obtained in California soils removed to Maryland. Kansas soil taken to California lost its power to produce a membrane in mannite solution, the *Azotobacter* flora became rather feeble, and the nitrogen-fixing powers of the soil were greatly reduced. The removal of the Kansas soil to Maryland increased the vigor of the *Azotobacter* and induced a higher fixation of nitrogen. The Maryland soil in California diminishes in nitrogen-fixing powers, but not in so great a degree as does the Kansas soil. This also happened when the Maryland soil was taken to Kansas. The bacterial flora of a soil is, therefore, dependent upon climatic conditions which affect many of the other properties of a soil.

#### RELATIONSHIP OF AZOTOBACTER TO NITRATE ACCUMULATIONS

The fact that certain spots in western cultivated soil were very rich in nitrates was first observed by Hilgard (76). This he attributed to the rapid nitrification of the organic matter of the soil in the warm arid climate of the West when the moisture limit was removed by irrigation.

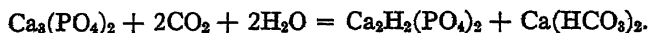
A number of years later Headden (69) noted these "nitre spots" in a number of Colorado soils, but he attributed it to the fixation of atmospheric nitrogen by the non-symbiotic bacteria which find in the western soils ideal conditions for growth and for rapid nitrogen-fixation. This conception has been further amplified by Headden (70, 71) and also Sackett (171). In this work it is assumed that the *Azotobacter* not only fix the nitrogen but also produce the nitrates. It has been proved, however, that these organisms do not produce nitrates (90).

Moreover, there are a number of other vital objections to this theory. (a) Lipman (125) has shown that for the fixation of the quantity of nitrogen which Headden maintains to have occurred, it would require from 1000 to 2000 tons of carbohydrates. There is no such visible supply of energy in these soils. True, many of these soils have a rich algae flora, but it has not been proved that this will furnish a sufficient supply of available energy. (b) The average amount of nitrogen fixed in 32 samples collected in the nitrate region was 7.4 mgm. (171), and the average nitrogen fixed in 31 samples of dry-farm alkali-free soil in Utah was 12.2 mgm. (55); yet there is no accumulation of nitrates in these latter soils. (c) The quantity of soluble salts occurring is often sufficient to stop the activity of all nitrogen-fixing organisms, if not to kill them (118). (d) The quantity of nitric nitrogen and of chlorine in any given "nitre spot" varies in the same spot from year to year or from period to period within a year (181). (e) The country rock adjacent to the nitrate accumulations, and which has contributed to the soil formation contains abundance of nitrates to account for the accumulations noted (182). (f) Soils having a similar physical appearance may be produced in the laboratory in the absence of bacteria. Because of this, we must conclude that the accumulation of nitrates in spots in western soils have their origin as do other accumulations of soluble salts found in the soil and not in the fixation in place by bacterial activity.

#### THE ACTION OF AZOFIERS ON PLANT-FOOD

It is quite evident that *Azotobacter* in their metabolism transform soluble inorganic soil constituents into either soluble or insoluble organic forms. This is especially true of phosphorus which is found in the ash of these organisms in such large quantities. The phosphorus, on the death of the organism, would be returned to the soil in a readily-available form, for Stoklasa has found that 50 per cent of the nitrogen of these organisms is nitrified within six weeks, and there is no reason for believing that the phosphorus would be liberated much more slowly. Then there is the possibility that many of the constituents of the bacterial cell may become available through the action of autolytic enzymes without the intervention of other bacteria (126).

It is further evident that an organism, which possesses the power when growing under appropriate conditions of generating 1.3 times its own body weight in carbon dioxide during 24 hours (185), must greatly change the composition of the media in which it is growing. Water charged with carbon dioxide is a universal solvent and will attack even ordinary quartz rock. Granite and rocks related to it are rather quickly attacked with the liberation of potassium and other elements. Likewise, it would act upon the tricalcium phosphate of the soil with the formation of more readily soluble phosphates, for this substance is four times as soluble in water charged with carbon dioxide as it is in pure water.



Moreover, the nitrogen-fixing organisms form among other products formic, acetic, lactic, butyric and other acids. The kind and quantity of each depends upon the specific organisms and upon the substance on which they are acting. These substances are sure to come in contact with some insoluble plant-food which may be rendered soluble, for they have a high solvent power for the insoluble phosphates (180). The resulting salts of calcium would be further attacked by bacteria with the formation of calcium carbonate (54).

Whether these processes will give rise to an increase in the water-soluble plant-food of the soil will depend upon whether the products of the second, the analytic reactions, exceed the products of the first, the synthetic reactions. We must not lose sight of the fact that, although many of the organic phosphorus constituents may not be soluble in pure water, they may be more available to the living plant than are the constituents from which they were at first derived through bacterial activity.

This being the case, we may expect to find variations in the results reported from laboratory tests. Stoklasa (184) found that bacterial activity rendered the phosphorus of the soil more soluble, whereas Severin (176) in his early work found the opposite to be true. Others have found that the solvent action of bacteria for insoluble phosphates is in direct proportion to the acid secreted by the organism (172).

In a later work, Severin (177) obtained different results. He used three soils—one sterile, a second sterilized and inoculated with pure cultures of *Azotobacter*, and a third sterilized and inoculated with cultures of *B. radiculicola* and *Azotobacter*. The solubility of the phosphorus increased 8 to 14 per cent over that in the sterile soil. The acid-producing organisms, because of the acid secreted and their intimate contact with the soil particles, possess the power of dissolving silicates (5). Moreover, since arsenic greatly stimulates nitrogen-fixation, there is a relationship between this increased bacterial activity and the form and quantity of phosphorus found in a soil (57).

The following results were obtained as an average of a great number of determinations. The addition of 16.0 mgm. of arsenic to a soil in the form of lead arsenate increased the nitrogen fixed in unit time 5.6 mgm. per 100 gm. of soil. It increased the water-soluble phosphorus in that soil 0.07 mgm., the 12 per cent hydrochloric acid-soluble phosphorus 5.8 mgm., and the organic phosphorus in the soil 1.3 mgm. per 100 gm. of soil. Now it is known that arsenic increases the activity of these organisms when applied to them even in the presence of an abundance of available phosphorus. It seems reasonable, therefore, to conclude that the excessive bacterial activity had slightly increased the water-soluble, the acid-soluble and the organic phosphorus of the soil.

Although the metabolic activity of *Azotobacter* gives rise to large quantities of phosphate solvents, yet these organisms transform phosphorus into organic phosphorus compounds less rapidly than do the ammonifiers (189).

## SOIL INOCULATION

High hope was entertained that the nitrogen problem in agriculture had been solved, when Caron (25) announced that he had prepared a culture of bacteria which would enable non-leguminous plants to utilize free atmospheric nitrogen, provided certain precautions were observed. Many of the results which he reported on pot experiments were clearly in favor of the inoculated plant. Stoklasa (183) was one of the first to study in detail the commercial preparation "alinit" which was placed on the market as a result of Caron's work. His findings were fully as favorable as Caron's, but the work of others soon demonstrated that alinit neither in the laboratory nor in the field had the ability to fix nitrogen. When Beijernick discovered the free-living aerobic nitrogen-fixers, the hope that soil inoculation may be so perfected that it would be beneficial to crops was revived, and since that time many investigators have attempted to inoculate soil in order to increase its crop-producing powers, but usually with negative results. Stoklasa (188) has made great claims for soil inoculation. He found that soils, inoculated with *Azotobacter chroococcum* and adequately supplied with carbohydrates and lime, showed an increase in the number of nitrogen-fixing organisms, and also an increased yield both in quantity and quality of the crop. Stranak (191) also obtained a pronounced increase in the production of beets, grain, and potatoes on inoculating with *Azotobacter*.

There may be a decrease in the crop during the first year when carbohydrates and *Azotobacter* are added to the soil with a marked increase in crop during the second and third year. Even then, the soil may be left richer in nitrogen than it was at first.

*Effect of dextrose and sucrose on the productiveness and nitrogen content of the soil (96)*

CARBOHYDRATE ADDED PER 100 GM. OF SOIL	CROPS OBTAINED				TOTAL NITROGEN REMAINED IN CROP	TOTAL NITROGEN LEFT IN SOIL, SPRING OF 1906	NITROGEN AS NITRATES
	Oats, 1905		Sugar Beets, 1906				
	Dry matter	Yield of nitrogen	Dry matter	Yield of nitrogen			
					gm.	per cent	parts per million
None.....	100.0	100.0	100.0	100.0	0.5914	0.093	10
2 per cent dextrose.....	32.8	62.5	186.0	190.0	0.6814	0.105	17
2 per cent sucrose.....	33.3	58.7	179.0	195.0	0.6800	0.105	15
4 per cent sucrose.....	37.7	78.1	283.0	339.0	1.0092	0.119	37

It is often the case that the addition of starch to a soil during the first year retards plant growth. This injurious action (40) may be due to the augmented bacterial activity in the soil brought about by the carbohydrates which injure the roots of the plant by withdrawing oxygen and by forming hydrogen sulfide in the deoxygenated atmosphere of the soil through the reduction of sulfates by the bacteria.

The effect produced by the carbohydrate applications also varies with the season (64). If applied to the soil in the spring when the soil temperature is low and when other bacteria are more active than *Azotobacter*, the results are that they rapidly multiply and compete with the higher plants for the limited available plant-food. If, however, the carbohydrates are applied in the autumn directly after the removal of the crop, when the soil is warm, *Azotobacter* are active, with the result that sufficient nitrogen is fixed to produce an increased crop the following season.

If the same quantity of carbohydrates per unit of nitrogen fixed be required by the organism under natural conditions, as are found necessary in laboratory experiments, enormous quantities would be required for the fixation of any considerable quantity of nitrogen; but it is possible that in the soil they are more economical (100) with their energy or they may live in symbiosis (106) with other organisms which furnish them part of their carbon.

Many workers have noted either no effect (33, 123) or even a detrimental influence (124, 196) when soils are treated with the carbohydrates and then inoculated with *Azotobacter*. This may be due in a great measure to any or all of the following factors: (a) absence of a suitable environment, as temperature, moisture, aeration, food and alkalinity; (b) absence of a suitable host from which *Azotobacter* may obtain part of its carbon; (c) injurious effects due to the decomposition products of the carbohydrate added (96).

There is considerable interest in the work of Bottomley (17) who uses bacterized peat, or humogen. The bacterizing process consists of three stages: (a) treatment of peat with a culture solution of the special "humating" bacteria and an incubation of it at a constant temperature for a week or ten days, during which period soluble humates are formed; (b) destruction of the humating bacteria by sterilization with live steam; (c) treatment of this sterilized peat with mixed cultures of nitrogen-fixing organisms—*Azotobacter chroococcum* and *Bacillus radicicola*—and an incubation at 20°C. for a few days, after which it is ready for use.

Theoretically, there is much in this process which recommends it, for there is no abrupt change in environmental conditions for the organism added, as would be the case when added from laboratory culture. Moreover, they are added in enormous quantities and with a source of carbon which is not far different from that found in the soil. Russell (170), however, after carefully reviewing all of the experimental evidence on the subject, concludes: "There is no evidence that humogen possesses any special agricultural value. There is not the least indication that it is 50 times as effective as farmyard manure, to quote an often repeated statement, and there is nothing to show that it is any better than any other organic manure with the same nitrogen content." Furthermore, he concludes that there is no definite evidence that "bacterization" really adds to the value of peat.

The conclusion is evident that soil inoculation, in order to be successful, must be accompanied by the rendering of the physical and chemical properties

of the soil ideal for the growth of the specific organisms to be added. A few organisms placed in a new environment already containing millions can never hope to gain the ascendancy over the organisms naturally occurring in the soil, for they have been struggling for countless generations to adapt themselves to the environment and only those which are fitted have survived. The problem becomes even more complicated when we recall the findings of Lipman that the bacterial flora of a soil is in many cases entirely changed by climatic conditions. On this account, it would appear that ever to make soil inoculation a success the chemical, physical, and even the biological condition must be made suitable for the growth of the specific organism added. Furthermore, strains of the organism must be used which have been evolved under similar climatic conditions.

#### SOIL GAINS IN NITROGEN

It is well established that many forms of microscopic organisms possess the power of fixing nitrogen either when grown alone or in combination with other organisms of the soil. Many of these have been obtained in pure culture and their morphology and physiology carefully studied. The most favorable conditions for their maximum nitrogen-fixation in pure cultures in liquid solutions have been accurately determined. Some of the conditions requisite for their activity in soils are known, but on this phase of the subject there are many gaps in our knowledge and much work must yet be done before we can state definitely the part which they play in the economy of nature and before we can say which are the very best methods for increasing their usefulness. Nevertheless, it is interesting to consider the results obtained by a few workers.

Berthelot's early laboratory experiments (13) led him to believe that sands and clays may fix in a year from 75 to 100 pounds of nitrogen to the acre. In two exceptional instances he noted that nitrogen was fixed by sands at the rate of 525 pounds and 980 pounds an acre, but soils which contained fairly large quantities of nitrogen never made markedly rapid gains.

Thiele (193), on the other hand, maintained that while there is no doubt that *Azotobacter* possessed the power of fixing free nitrogen, under laboratory conditions, yet it is not certain that conditions would be such in soils for any gain of nitrogen due to the activity of these organisms. We have already seen, however, that the *Azotobacter* do not require as high a temperature for nitrogen-fixation in soil as he thought necessary. It is also certain that in most arable soil the temperature is sufficient during a large part of the year for a fairly rapid nitrogen-fixation by bacteria.

Krainskii (106) thinks that even better results should be obtained in soils than in pure solutions, for there the nitrogen-fixers grow in symbiosis with autotrophic organisms which form organic compounds available to the *Azotobacter*. In soils the nitrogen fixed is rapidly removed by other plants, because

of which the slowing-up process which becomes perceptible so early in laboratory experiments should not occur.

In addition to an optimum temperature and moisture content of the soil, the *Azotobacter* are dependent upon a supply of carbon for energy and inorganic nutrients for the building of cell protoplasm. Unfortunately, it is too often the case that under natural conditions those soils which are deficient in nitrogen are also lacking in available carbon, and especially in phosphorus, which are so essential for rapid nitrogen-fixation. Then there are the technical difficulties which the chemist encounters in determining the gain or loss of nitrogen which occurs in soils under natural conditions and which may be attributed to non-symbiotic nitrogen-fixation.

There are, however, several cases in which this has been measured with a fair degree of accuracy.

Lipman (198), in pot experiments carried on with a soil containing about 5000 pounds of nitrogen per acre-foot of soil, found a gain of more than one-third this amount in two short seasons. Much of this must be attributed to non-symbiotic nitrogen-fixation. To these soils had been applied solid and liquid manure, which furnished to the organisms readily-available supplies of energy and various necessary inorganic constituents. This fixation was not nearly so rapid where legumes were turned under as green manures.

Koch found a gain of from 0.093 per cent to 0.019 per cent in soil nitrogen during two seasons which must be attributed to non-symbiotic nitrogen-fixation. In addition to this there was a threefold gain in the nitrogen content of the crops—oats, buckwheat, and sugar beets—which must also be attributed to the action of *Azotobacter*.

Hall (63) noted an annual gain of 100 pounds of nitrogen on Broadbalk field at Rothamsted and 25 pounds on Grescroft field. He feels that much of this gain must be due to the action of non-symbiotic bacteria. Lipman (199) points out that the actual gains of nitrogen are even greater, for this does not take into consideration the various losses which are sure to occur even under the best of conditions. Hopkins (82) takes the stand that the apparent gain is due in a large measure to drifting dust and plant residues coupled with the difficulty of obtaining representative samples of soil at the two different periods. When all of these factors are considered the evidence points to a gain of nitrogen through bacterial activity.

The analysis of a great number of soils in Utah (56) showed that the average nitrogen content of the soil which had grown wheat and other non-leguminous plants for from 20 to 50 years was 0.2009 per cent, whereas adjoining virgin soil on the average showed only 0.0984 per cent of total nitrogen. The evidence is very strong that considerable nitrogen has been added to these soils by microscopic organisms, for:

(a) In nearly every case the cultivated soil fixed much more nitrogen in the laboratory than did the virgin soil. This was the case when the soil was incubated with or without the addition of carbonaceous material.

(b) There is a richer nitrogen-fixing bacterial flora in the cultivated than in the virgin soil.

(c) The conditions of moisture, alkalinity and food constituents in the soil were ideal for rapid nitrogen-fixation, and the temperature of the soil was high enough during a considerable part of the year for the growth of *Azoobacter*.

(d) The cultivation of the soil would increase aeration and available phosphorus in the soil.

(e) The large quantity of plant residues would act as a supply of carbon which is readily rendered available by the soil's rich flora of cellulose ferments. If these soils had produced a wheat crop every alternate year and all of the nitrogen which had been added to the soil without loss from leaching or bacterial activity taken by the crop, it would have necessitated the addition of 25 pounds an acre yearly, which is evidently the very minimum which can be attributed in these soils to non-symbiotic nitrogen-fixation.

Eighty different samples of these soils were incubated in the laboratory for 21 days and the gains in nitrogen determined by comparing with sterile checks. The soils were incubated without the addition of anything except sterile distilled water. At the end of the period the average gain per acre for the cultivated soils was 202 pounds and that for the virgin soil was 92.

True, fixation would not continue long at this rate, for when the nitrogen content of the soil passed beyond a certain limit, decay bacteria increase rapidly (79), and in the struggle for existence they are able, with the advantage at their disposal, to suppress the more slowly growing *Azotobacter*, which would gain the ascendancy again only when the nitrogen of the soil became low.

Thus, there is an upper as well as a lower limit to the nitrogen content of the soil as far as bacterial activity is concerned, but by making the conditions for nitrogen-fixation as nearly ideal as possible we may maintain in a soil the upper and not the lower nitrogen content.

In conclusion, it may be stated that, although the part played by *Azotobacter* in maintaining the nitrogen of the soil has not been definitely measured, it is nevertheless an important factor. Hall (63) found it at least 25 pounds, Löhnis (134) 35.7 pounds, and the Utah Agricultural Experiment Station 25 pounds per acre annually. It is therefore, conservative to state, as has Lipman (125), that these organisms, under favorable conditions, add from 15 to 40 pounds of available nitrogen to each acre of soil yearly.

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## HYDROGEN-ION CONCENTRATION—SOIL TYPE—COMMON POTATO SCAB

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In a former paper (9) we called attention to a characteristic difference of hydrogen-ion concentration shown by the extracts of soils of the types Caribou loam and Washburn loam. These types, which have similar texture, are derived from the same glacial till, and seem to owe their striking dissimilarity to local topographic differences, which affect particularly the drainage. In the Soil Survey of the Caribou Area, Maine, by Westover and Rowe (22), reference is made to the fact that potatoes grown on the Washburn loam are in general very subject to common scab, whereas potatoes grown on the Caribou loam (and Caribou silt loam) are of fine quality and are produced in notable yield. We found the Caribou loam more intensely acid than the Washburn; though both types are acid, as is locally recognized by the use of litmus paper. The Caribou loam is characterized by the yellowish-brown color of the surface soil and even brighter subsurface soil (indicative of good drainage); whereas the surface of the Washburn loam is dark gray to black in color, and is underlain by a light gray to a dull yellow and gray mottled subsurface soil, the latter being very characteristic of poorly-drained soils. Other differences have already been described (9).

In connection with a study carried on coöperatively between the United States Department of Agriculture and the Maine Agricultural Experiment Station, we collected during the season of 1917 and examined a large number of samples of these two types, including also soils intermediate in character between the Caribou and the Washburn loams, or otherwise not typical of either soil type. A search was made for soils exceptional in their behavior toward the scabbing of potatoes, and some soils of the Caribou series (not typical Caribou loam) were found producing scab, as well as three samples of Washburn loam from which clean or only slightly infected potatoes were harvested.<sup>1</sup>

In every case except when otherwise noted, the presence or absence of scab was determined and the sample taken at the time of digging, and the sample of soil was therefore known to represent the soil condition in question. It

<sup>1</sup> Very little or no powdery scab was found in Aroostook County in the summer of 1917, and all reference to scab in this paper applies to the common (corky) scab, actinomycosis.

was not possible to determine the hydrogen-ion concentrations on the soil fresh from the field, and the soils were air-dried in a place free from laboratory fumes. The surface soil was sampled, down to the change of color, or the average plow depth, the average depth being 7 or 8 inches.

#### ELECTROMETRIC DETERMINATIONS

The electrometric determinations were made by the use of the electrode vessel previously described (7). This vessel was designed especially with regard to the nature of the material to be examined and the difficulties to be overcome, and the recommendations of Hasselbalch (11) and Cumming and Gilchrist (6) were followed. This vessel avoids the error due to the entrance of potassium chloride into the soil suspension when the liquid contact is made by means of a tube of agar with potassium chloride (13). A high-grade potentiometer was used in connection with a galvanometer. The low-resistance galvanometer coil regularly supplied was not sufficiently sensitive when used for hydrogen electrode chains of high resistance. Another coil, having a resistance of about 400 ohms, was substituted by the manufacturers, and was very satisfactory both for soil work and for solutions of good conductivity in the Clark electrode vessel (1). Commercial switches and heavily insulated "fixture" wire were used.<sup>2</sup>

In figure 1 is shown the electrode vessel together with the electrode in position, the soil and water mixture, and the approximate location of the junction between the saturated potassium chloride solution and the soil extract. Mechanical shaking was provided. During the shaking the vessel turned from an inclination of 3° to the horizontal to an inclination of about 33°; the presence of the soil mass prevented the rising of bubbles of hydrogen into the soil extract in the neck of the vessel. The shaker consisted of a horizontal cross-piece (passing through  $\pi$  perpendicular to the vessel as shown) actuated by an eccentric. Two metal arms were fastened to the cross-piece, the arms were covered with rubber tubing, and the vessel was laid lengthwise along the arms and fastened with strong rubber bands. The electrode vessel had a capacity of about 65 cc., measured from the stopcock to the brim (for a vessel of different dimensions a different quantity of soil suspension would have to be chosen, in order to secure satisfactory agitation of the mass with hydrogen). A small motor was used to drive the shaker, the speed being reduced in two

<sup>2</sup> The least satisfactory part of the electrical system formerly used (12) was not the much-abused capillary electrometer, but the voltmeter, which is not graduated in enough subdivisions for precision and which also requires calibration. (The one in question required a correction of about 1.5 per cent.) The capillary electrometer gave very good service. The capillary was not so fine as "thermometer tubing," and the instrument was short-circuited through soldered connections and platinum contacts, and put in good condition once in a while by applying for a few minutes an electromotive force of about 0.8 volt in the direction to make the mercury contract in the capillary (19). Electrical shielding (23) was necessary with this former system.

steps by light pulleys so that the vessel was rocked at the rate of 70 to 90 complete swings per minute.

Hydrogen was generated from caustic potash solution with iron electrodes (somewhat better with a platinum anode and a nickel cathode) using the lighting circuit of 220 volts with a lamp-bank in series. Current for coating and treating the electrode was taken off from the terminals of the hydrogen generator. The coating bath was prepared by adding a trace (about 0.03 per cent) of lead acetate to the commercial solution (3 per cent) of palladium chloride; from time to time the volume was restored by addition of distilled water, or the color restored by adding more palladium chloride. The cleaning bath was dilute sulfuric acid. Platinum anodes about 1 inch square were used for both baths. *The palladium coating was not appreciably scoured off*

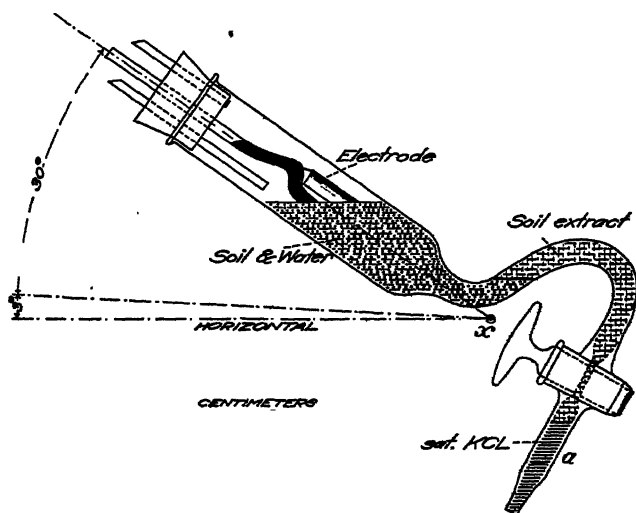


FIG. 1. THE FILLED ELECTRODE VESSEL

by the action of the soil, but it was *always renewed, after complete removal of the previous coating, for each determination*, in order to exclude the possibility of an accumulative poisoning of the electrode. The time required for (lightly) coating the electrode was about 5 seconds, and after thorough washing in a stream of distilled water the electrode was then put into the cleaning bath and hydrogen liberated from the electrode for 5 to 10 seconds, after which the electrode was again thoroughly washed.

The hydrogen electrode is shown in figure 2. A sheet of platinum was used which measured 2.5 by 3.3 cm. (An electrode 1 inch square also was satisfactory.) In constructing the electrode it was necessary to support the sheet of platinum at both top and bottom, and also to provide leads for the current at two opposite points in order to secure an even deposit of palladium black. This was accomplished by welding platinum wires at opposite ends

and sealing the wires into the glass tube, which was supplied with mercury. During the shaking the electrode was never entirely out of contact with the soil suspension.

The rest of the apparatus was simple. A glass T-tube was affixed to an upright piece of board. Through one arm of the T-tube saturated potassium chloride solution could be drawn through a stopcock; another arm (which was horizontal) could be connected with the end-tube *a* of the electrode vessel by means of a rubber tube; and the third arm (also horizontal) connected with a saturated potassium chloride calomel electrode (17) through a stopcock greased at the edges, but not in the middle (1). After the apparatus was once set up it was unnecessary ever to open this last-mentioned stopcock.

The course of the determination is as follows. Fifteen grams of the air-dried soil are introduced into a test-tube 17 by 3 cm. containing 30 cc. of distilled water, the tube is closed and shaken vigorously by hand 50 times, and permitted to stand 20 minutes or longer for sedimentation. (A number of samples are thus treated at once.) The previous coating of palladium black is removed from the electrode by placing this in a beaker of strong nitric acid (or a cold mixture of nitric and hydrochloric acids). While the coating is



FIG. 2. THE HYDROGEN ELECTRODE

being dissolved the electrode vessel is cleaned and filled. The neck of the electrode vessel, including the bore of the stopcock, is filled with the comparatively clear soil extract; the stopcock is closed and the end-tube *a* is filled with saturated potassium chloride solution by means of a capillary pipette; the rubber tube leading to the calomel electrode is filled with potassium chloride solution and slipped over the end-tube *a*, avoiding the entrance of air-bubbles; and the vessel is fastened to the shaker. The rest of the soil mass is well agitated and poured into the vessel. The bare electrode is coated and cleaned as described above, the electrode is put into the rubber stopper, and this stopper, which fits well and carries tubes for the entrance and exit of hydrogen, is put into the electrode vessel, as shown in figure 1. The entrance and exit of hydrogen is controlled with light pinch-cocks. A generous volume of hydrogen (about 150 cc.) is rapidly swept through the space over the soil mixture, the current of hydrogen stopped by closing the exit, the flexible wire leading to the potentiometer is put into the mercury tube of the electrode, and the electrode vessel is shaken for 5 minutes, the stopcock being closed. Without stopping the motor the liquid contact can be made and the potential measured. To effect liquid contact the rubber tube connecting the vessel with the calomel electrode is pinched, forcing potassium chloride solution through the stopcock in the arm of the T-tube back into the reservoir, this

stopcock is closed, and the stopcock of the electrode vessel is opened, drawing some of the soil extract down into the end-tube *a* of figure 1. The boundary formed is not sharp like the line shown in figure 1. From the measured difference of potential and the room temperature, which was not permitted to change excessively, the hydrogen-ion exponent was calculated from the figures given by Michaelis for the saturated electrode (17). The calomel electrode has been checked many times against "standard acetate" and Clark and Lubs' buffer mixtures.

If the electrode vessel is not shaken during the measurement the potential is not constant, but becomes numerically greater, sometimes so fast that any measurement would be a forced one. This is due to the presence of nitrates (21, 10) (and possibly other oxidizing agents). In order to see whether a substantially correct potential is obtained (when shaking is continuous), the stopcock was closed and the vessel shaken 5 minutes longer, liquid contact made as before and the potential determined, the vessel still being shaken continuously. The changes of potential due to the extra 5 minutes' shaking are recorded in table 1.

TABLE 1

*A frequency table showing the number of times each change of potential was observed. (For 58 determinations the mean change was -0.9 millivolt, the minus sign signifying a fall of potential)*

	CHANGE OF POTENTIAL IN MILLIVOLTS												
	-5.5	-4.0	-3.5	-3.0	-2.5	-2.0	-1.5	-1.0	-0.5	0.0	0.5	1.0 *	1.5
Number of times each change was observed...	1	2	1	1	1	5	5	12	11	13	5	0	1

Three larger changes were seen (+8, -5, and -12 millivolts) and the results were disregarded and the determinations of hydrogen-ion exponent repeated with better results. The changes shown on the repetition are included in table 1 (the first value, -5.5, is one of these). Table 1 shows how "constant" the potential may be expected to be. The second measurement of potential is a simple control. If the vessel were not shaken during the measurements, and a final potential reading were taken after the potential seemed to become constant, the results in some cases would be quite erroneous, and it would appear that in general such results would be more or less in error.<sup>3</sup>

<sup>3</sup> Kappen applies the hydrogen electrode to the extract separated from the soil, as appears from the following, taken from an abstract (14). "Only soils reacting acid to litmus raised the hydrogen-ion concentration (H-Zahl) of the extract (Schuettelwasser), soils reacting weakly or neutral decreased it, with one exception. The presence of solid soil particles in the extract increased the hydrogen-ion concentration." In the preliminary work in this laboratory it was observed that the hydrogen-ion concentration of the extract comparatively free from soil particles was less acid, but also indefinite and meaningless, and this was the reason for operating on a suspension and for shaking the electrode vessel during



## COLORIMETRIC DETERMINATIONS

Since the former comparison of methods (7) the colorimetric method for the determination of hydrogen-ion concentration has been improved, and for the bacteriologist, standardized, by Clark and Lubs (3). Their method was used in the present work, 5 cc. of solution to be examined and 5 cc. of buffer mixtures being used instead of 10. The comparator was used throughout and the yellow screen light recommended by them was used for measurements with brom-cresol-purple and brom-phenol-blue. The use of dipropyl red (2) was discontinued because the colors of the standards faded rapidly, though a few measurements with it gave excellent results. In the former work (7) all the indicators were used in alcoholic solution. In this work methyl red was used as the free indicator in alcoholic solution, and the other indicators in water solution as the monosodium salts. This choice is in accordance with the recommendations of Clark and Lubs and with their implied doubt as to the suitability of methyl red as the sodium salt in buffer-poor solutions, and is theoretically better than the use of all the indicators in the free state, or all as the monosodium salts, since methyl red is a weak acid, whereas the sulfone-phthalein indicators have a highly ionized hydrogen atom (15). We used the sodium salt of methyl red in our previous work on the Caribou and Washburn loams, without electrometric control, and have found that the exponents so obtained with that indicator are about 0.3 to 0.4 too high. Some of the trouble was due simply to the use of the sodium salt, and some probably to impurities. Dr. Lubs states that the commercial product is likely to contain acetate. He kindly gave us some which he had synthesized and recrystallized from toluol, and this preparation, dissolved in redistilled alcohol (and water added), was used for the measurements reported, with excellent results. We have not determined whether such a solution will keep indefinitely. The monosodium salts of the sulfone-phthalein indicators were prepared by treating the free indicators with standard sodium hydroxide solution. Clark and Lubs use 1.1 equivalents of alkali for brom-phenol-blue and brom-thymol-blue, and in the case of the brom-cresol-purple, they use 1.5 equivalents (4). We were not able to get the last-named into solution in 1.5 equivalents of sodium hydroxide with moderate stirring or shaking, unless the mixture was heated, in which case the substance seemed to undergo slight decomposition, as the colors yielded by such a preparation in buffer mixtures were not quite so deep and brilliant as those produced by the same quantity of indicator brought into solution with alcohol. This decomposition was apparently minimized by the use of gentle heat applied for a short time to the indicator and sodium hydroxide solution in small volume. The quantities of

the measurements. The cause of the difference is the presence of nitrates in a solution weak in buffer action (21, 10). In some cases the ammonia formed locally about the hydrogen electrode is sufficient to change the reaction of the whole fluid, so that the change can be detected with indicators. So far as is known, extracts of normal soils cannot be measured electrometrically, but only by the indicator method.

N/10 sodium hydroxide solution required to furnish the given equivalents for the three indicators, in the order named, were 1.64, 1.76, and 2.78 cc., for decigram portions of the indicators.

When comparing the colors it is important to avoid using a given indicator for too great a range of hydrogen-ion exponent. When a highly turbid or colored extract is being examined, the mere fact that no difference can be seen in color, when indicator has been added and comparison is made with the colors of the buffer standards, does not prove that the value of the exponent has been located; on the contrary, it is absolutely necessary that the color is seen to differ definitely when either a more acid or a less acid standard is used for the comparison. If this is not done, it is easy to locate the value at the end or near the end of the range of a given indicator, when as a matter of fact the true value lies entirely outside the range. The useful ranges of the various indicators are somewhat shorter for colored or turbid solutions than for clear solutions.

The indicator solutions were of the same strength as recommended by Clark and Lubs, namely 0.04 per cent for the three sulfone-phthalein indicators, and 0.02 per cent for methyl red, which is prepared by dissolving 0.1 gm. in 300 cc. of redistilled alcohol and diluting to 500 cc. with distilled water.

The procedure for the examination of soil was as follows. Fifteen gm. of the air-dried soil was added to 30 cc. of distilled water, in a large test-tube, the tube was closed and vigorously shaken by hand 50 times (just as for the electrometric determinations), the mixture was centrifuged, and the extract drawn off in a pipette provided with a rubber tube and mouthpiece. Five-cubic-centimeter portions were distributed into test-tubes, two or three drops of indicator added, and the color compared with the colors of the buffer mixtures containing the same quantities of indicator, a comparator being used for the comparison. One 50-cc. pipette and 6 test-tubes were used for the soil extracts; they were scrupulously clean, and rinsed well with distilled water. The rinse-water was shaken out of the test-tubes, and allowed to drain out of the pipette; it was not necessary to dry the glassware, since small variations in the volume produce no sensible error.

It is very convenient to have a selection of test-tubes, chosen so that a given volume (say 10 cc., delivered from an automatic pipette) corresponds to nearly the same height in all the tubes, say between 8 and 8.5 cm. The portions of buffer or of soil extract are then measured by filling up to a given height, using a tube for comparison containing the desired volume. Such a method of *constant height* leads to no greater error than the method of *constant volume*.

#### COMPARISON OF METHODS

A good though not perfect agreement was previously found between colorimetric and electrometric results (7). Such an agreement is of considerable

importance, because various objections could be brought against either method, which might appear to have force if either method had been used alone, without ever having been supported by the other. Against the colorimetric method it might be urged that the color changes were due to adsorption by the small quantity of colloidal material present in the unfiltered though centrifuged extracts, just as the action of soil on litmus paper was formerly rather widely attributed to adsorption. Against the electrometric method, on the other hand, it might be objected that strictly constant potentials would not be observed if the classical (unshaken) electrode vessel were used. Shaking the electrode vessel during the measurements has not been practised in purely physico-chemical investigation, but is a device of the biological chemist to reduce errors admittedly inherent in the measurement of blood, sea-water, and also soils. It is furthermore regarded by everyone as an absolute necessity to avoid the soiling of the electrode with the fingers, lest the electrode be poisoned and lose its characteristic catalytic property, and though this precaution was of course taken, yet it seems almost futile when it is considered that the electrode is plunged into a soil suspension. Such a poisoned electrode might give very erroneous results, and might seem to show a constant potential (especially if the potential can be observed only to centivolts) when approximate equilibrium was far from being attained.

It is therefore of interest to compare again the results obtained by the two methods, obtained by the technique described above, which embodies improvements in the colorimetric procedure.

Table 2 affords a comparison between the results given by the two methods. Fifty-seven soils were studied, including some samples not discussed in this paper, and 88 colorimetric determinations made. The results are compared by assuming the electrometric result to be correct, and tabulating the errors of the colorimetric results. To a certain extent this is unfair to the colorimetric method, since any comparison of the two methods includes the error of duplicating the sample. As judged by duplicate colorimetric determinations with the same indicator, the error of sub-sampling from a sample collected from the field, air-dried, and mixed well but without special precautions, may cause an error of about 0.1 in the value of the hydrogen-ion exponent, which would correspond to an error of about 6 millivolts. It will be seen that the difference between colorimetric and electrometric results is at the most 0.3 exponent, or about 18 millivolts, and not very often so large. Only a few electrometric determinations have been done in duplicate; those so done have given results agreeing within 2 or 3 millivolts, except in those cases already mentioned above, where the potential varied excessively during the second shaking. It has recently been reported almost impossible to obtain duplicates electrometrically agreeing within 20 millivolts (0.02 volt) (20). This was taken to indicate lack of uniformity of mixing, but may rather indicate an electrometric procedure faulty in some detail.

The average error for all indicators was 0.08 in the value of the exponent, and the mean error was  $-0.001$ . In almost all cases the results obtained by the two methods agree within what the experimenter feels to be the uncertainty of the color distinctions. This uncertainty depends on the indicator used and on the color and turbidity of the soil extract. The color standards employed differ from one another by intervals of 0.2. The two methods may be said to give the same result, within the experimental error.

The data of table 2 dispose of such objections as have been mentioned above, but it does not follow that any and all procedures for measuring hydrogen-ion concentration are equally applicable to soil, and it would seem

TABLE 2  
*Errors of the colorimetric determinations*

ERROR	NUMBER OF TIMES THE ERROR OCCURRED				
	Brom-phenol-blue	Methyl red	Brom-cresol-purple	Brom-thymol-blue	Total for all the indicators
0.30	0	0	1	0	1
0.25	1	1	0	0	2
0.20	1	0	2	0	3
0.15	1	0	3	3	7
0.10	2	1	4	5	12
0.05	6	7	2	0	15
0.00	0	11	2	2	15
-0.05	0	5	3	1	9
-0.10	0	4	8	0	12
-0.15	0	2	4	0	6
-0.20	0	0	2	0	2
-0.25	0	0	1	0	1
-0.30	0	0	3	0	3
Number of determinations.....	11	31	35	11	88
Average error.....	0.05	0.05	0.10	0.09	0.08
Mean error.....	+0.05	-0.01	-0.04	+0.08	-0.001

advisable, if only one of the methods can be used, to adopt a procedure very similar to one which has been tried out, and not to vary the procedure without means for checking modifications. The colorimetric method has recently been applied to filtered soil extracts (18). Though we have not exhausted the possibilities, our results with soil extracts filtered through filter paper have been incorrect and variable, according to the brand and treatment of the paper.

In addition to the observations summarized in table 2, we have often measured the same soil extract by means of two different indicators, and have thus been able to check one indicator against another without introducing any error of sampling. These checks have been most satisfactory. Not only have

the indicators mentioned above been checked against one another in this way, but also phenol-sulfone-phthalein has been checked against brom-thymol-blue. When the hydrogen electrode is not available, this method of checking the suitability of the indicator solutions is very useful and important.

#### EFFECT OF DILUTION ON THE REACTION

Sharp and Hoagland (21) examined electrometrically soil suspensions prepared with various ratios of water to soil, the lowest ratio in any case being 2 to 1 (2 cc. of water per gram of soil). They concluded that the change of hydrogen-ion concentration was small compared with the wide variations in the ratio. The ratio cannot be reduced much below 2 in electrometric determinations; in colorimetric determinations, however, good duplicates can be obtained with a ratio 1 to 1. We stated (9) that the average difference obtained by varying the ratio from 1 to 1 to 2 to 1 (through an error, "2 to 2" was printed) was 0.14 in the value of the exponent for 9 soils. This was unexpectedly large, and on extending the experiment to a larger number of soils

TABLE 3

*A frequency table showing the effect on the hydrogen-ion exponent of changing the ratio of water to soil from 1 cc. of water per gram of soil to 2 cc. per gram*

	DIFFERENCE OF EXPONENT DUE TO DILUTION								
	-0.15	-0.10	-0.05	0.00	0.05	0.10	0.15	0.20	0.25
Number of times each difference occurred.....	7	14	3	11	9	3	6	6	3

we have not been able to confirm so large a difference. We have studied 43 soil samples, showing exponents from 4.5 to 6.2, and have made 62 measurements of the difference, using methyl red and brom-cresol-purple. Table 3 shows the values thus obtained for the difference of exponents caused by varying the ratio of water to soil from 1 to 1 to 2 to 1. A positive difference means that the extract was less acid when more water was used.

The differences are distributed like errors about a mean value of +0.04, which seems to be a real value, not an error itself. (There was no apparent correlation between the actual values of the exponent and the difference caused by dilution.) We therefore think, with Sharp and Hoagland, that the error caused by adding to the soil the quantity of water actually necessary for the determinations is probably not serious. Plummer (20) has recently examined soil suspensions and also the soil solution obtained by the oil-pressure method. He concluded that the reaction of the soil solution so obtained is qualitatively the same as that of the suspension, but different quantitatively. The quantitative differences found are in some cases rather large. The oil-pressure method requires considerable time for the collection of the solution; in the experiments under discussion this time was from 2 to

4 days. In the absence of air anaerobic processes might in some cases be set up, and the control experiments did not exclude the possibility that anaerobic processes occurred involving changes of reaction.<sup>4</sup>

#### HYDROGEN-ION EXPONENT AND THE OCCURRENCE OF POTATO SCAB

We have already indicated that the relative freedom of the Caribou loam from potato scab may be due to its greater hydrogen-ion concentration (9) and that the acidity of the Caribou loam is often sufficiently intense to exert a restraining influence on the organism causing the common (corky) potato scab (8). The data to be presented in this paper are sufficient for a more searching inquiry into this relationship.

The hydrogen-ion exponents found electrometrically are given, together with the notes as to the occurrence of scab and the soil type, in table 4. The soils are numbered in order of decreasing acidity. As the exponent increases, the hydrogen-ion *concentration* decreases, neutrality being reached at about the exponent 7. The second decimal has little significance but has been retained for convenience in ordering.

A glance at table 4 shows a general correlation between hydrogen-ion exponent and the occurrence of potato scab, the more acid soils at the head of the list being free of scab, and the less acid or neutral soils at the foot of the list exhibiting scabby potatoes. With the exception of soil no. 9, the exponent for which is 4.9, and from which the potatoes were only slightly scabby, no scab is recorded until the exponent 5.16 is reached, at no. 20. From then on to 5.5 the scabbing is irregular. From the exponent 5.5 down through the rest of the soils, scabbing is present in every instance except in the case of no. 36, where the land has just been cleared and put into potatoes, and in which case the potato scab organisms had not had opportunity to spread through the soil. Three fields, as shown in the notes in table 4, yielded scabby potatoes in one portion of the field and clean potatoes on another portion; these differences being correlated with differences of hydrogen-ion exponent and also of soil type. The notes illustrate the characteristic freedom of Caribou loam from potato scab and the characteristic infection of potatoes grown on the Washburn loam, there being a few exceptions.

One of us has recently studied the growth of different strains of the potato scab organism at different hydrogen-ion exponents (8) and found that the exponent 5.2 represents a degree of acidity sufficient to delay or inhibit the growth; in some cases a slow but finally a good though abnormal growth occurred in a highly nutritive medium which had at the beginning the exponent 4.8, but it was shown that the acidity was being decreased in such cases during the growth, and the manner of growth indicated that successful growth depended on local decrease of acidity.

<sup>4</sup> We do not understand how the solutions could have been measured electrometrically, unless denitrification had occurred.

TABLE 4

*Hydrogen-ion exponents for the samples collected, with notes as to their type and the occurrence of scabby potatoes*

SOIL NUMBER	HYDROGEN-ION EXPONENT	OCCURRENCE OF COMMON SCAB	SOIL TYPE
1	4.50	No scab	Typical Caribou loam
2	4.64	No scab	Not Caribou loam but similar
3	4.76	No scab (see nos. 8 and 20)	Typical Caribou loam
4	4.77	No scab	Typical Caribou loam
5	4.80	No scab	Typical Caribou loam
6	4.80	No scab	Typical Caribou loam
7	4.81	No scab	Typical Caribou loam
8	4.90	No scab (see no. 20)	Typical Caribou loam
9	4.93	Potatoes only slightly scabby	Rather typical Caribou loam
10	4.98	No scab	Washburn loam
11	4.99	No scab	Typical Caribou loam
12	4.99	No scab	Caribou loam
13	5.00	No scab	Typical Caribou loam
14	5.03	No scab	Typical Caribou loam
15	5.04	No scab (see no. 39)	Fairly typical Caribou loam
16	5.09	No scab (rest of field scabby, see no. 37)	Caribou loam, not typical
17	5.10	No scab	Caribou loam
18	5.12	No scab	Typical Caribou loam
19	5.16	No scab	Caribou loam
20	5.16	Moderate infection of scab	Fairly typical Caribou loam, collected from same field as nos. 3 and 8, only 20 feet from no. 8.
21	5.18	No scab a month before digging	Rather typical Caribou loam
22	5.21	Little or no scab	Washburn loam
23	5.30	Potatoes very scabby	Caribou loam, not typical
24	5.38	No scab	Mucky phase of Washburn loam.
25	5.39	Potatoes slightly scabby	Washburn loam
26	5.44	Potatoes not scabby (new land)	Belongs with the Washburn loam, taken from a swale in Caribou loam
27	5.52	Slight infection of scab in spots of the field	Caribou loam, not typical
28	5.52	Medium infection of scab, in places only slight	Caribou loam, not typical
29	5.64	Very scabby potatoes	Caribou loam, not typical, possibly Caribou shale loam
30	5.68	Scabby potatoes	Washburn loam
31	5.75	Scabby potatoes	Washburn loam, not typical but somewhat like Easton loam
32	5.79	Scabby potatoes	Washburn loam
33	5.89	Very scabby potatoes	Caribou shale loam
34	6.00	Very scabby potatoes	Washburn loam
35	6.17	Potatoes very scabby the previous season (1916)	Washburn loam

TABLE 4—*Continued*

SOIL NUMBER	HYDROGEN-ION EXPONENT	OCCURRENCE OF COMMON SCAB	SOIL TYPE
36	6.22	No scab a month before digging (new land)	Washburn loam
37	6.24	Scabby potatoes	Washburn loam, from same field as no. 16
38	6.30	Very scabby potatoes	Mucky phase of Washburn loam
39	6.31	Scabby potatoes	Washburn loam, from same field as no. 15
40	6.45	Scabby potatoes	Washburn loam
41	6.48	Scabby potatoes	Washburn loam
42	6.63	Scabby potatoes	Washburn loam
43	6.66	Scabby potatoes	Washburn loam
44	6.69	Potatoes said to be always scabby	Washburn loam, not typical
45	7.14	Very scabby potatoes	Washburn loam, not typical
46	7.15	Very scabby potatoes	Washburn loam, not typical
47	7.21	Very scabby potatoes	Caribou shale loam

The limiting zone of hydrogen-ion concentration seems to be about the same in soil as in culture media, and this would appear to serve as a rough biological check on the methods used for determining the hydrogen-ion concentration of the soil.<sup>5</sup>

The above results, while obtained for soils of the types Caribou loam and Washburn loam, appear to have a more general application. A field of Norfolk sandy loam near West Norfolk, Va., has a record of clean potatoes, and the July crop of this year has been observed by Mr. B. E. Brown of this office to be very free from scab. Liming is not practised. We sampled this field in March of this year in six places, and found the hydrogen-ion exponent to be uniformly 5.1 in the surface soil. Two samples of sand from Florida were sent to the laboratory, which were much alike, aside from color, but one yielded clean potatoes and the other scabby ones. Mineral analysis by Dr. L. E. Wise showed the two soils to have very similar ultimate composition. The clean soil showed the exponent 5.0; the scabby soil, 6.3. We have collected some samples of Everglade muck and peat; a muck field, which is yielding good crops of clean potatoes, was sampled in four places, and showed the exponents 4.7, 5.0, 5.1, 5.1; whereas the peat field, which is in general a poor soil and in yielding a poor crop of scabby potatoes, showed the exponent 5.6. Two samples of soil from the same field were collected near Milford, Conn., one of which was limed in 1906, the other received no lime. The soils

<sup>5</sup> This does not mean that the use of air-dried soils is to be generally recommended, in fact fresh soils should as a rule be used where it is practicable. If a problem involves a critical zone of hydrogen-ion concentrations near the neutral point it would seem quite essential to use fresh soils. The sensitiveness of the hydrogen-ion exponent to carbon dioxide depends in general on the value of the exponent, and is comparatively small at the exponent 5 (17, p. 142, 13) though large in the case of soils at the exponent 7 (13).



were a reddish-brown loam, well drained and well suited to the growing of potatoes. The limed soil produced in 1906 very scabby potatoes, and again produced similarly scabby potatoes when next cultivated with potatoes in 1918; whereas the unlimed soil produced clean potatoes both years. The hydrogen-ion exponents were determined this year and were found to be 5.7 for the limed soil and 5.05 for the unlimed soil. If these results were incorporated in table 4, they would fit perfectly, and they confirm the conclusion, which can be based on the agreement between the soil observations and the culture medium experiments (8), that the results shown in table 4 are not limited to any particular soil type or condition, but are of general application.

It should be recalled that the organism causing scab on beets is supposed to be the same as that causing the potato scab (16) and the same relation should therefore apply also to beets. We have no direct evidence as to this.

#### HYDROGEN-ION CONCENTRATION AND SOIL TYPE

The relation between hydrogen-ion exponent and soil type is not easily discerned from the notes given in table 4, and can best be seen from the frequency diagrams shown in figure 3. From the upper diagram we can determine, for instance, that 9 soils of all those measured showed the exponent 5.0 (that is, from 4.95 to 5.15), and of these, one was a Washburn loam. From the lower diagram we see that eight Caribou loams showed the exponent 5.0, and of these eight, four were typical Caribou loams. The exponent 4.8 is characterized by the greatest number of typical Caribou loams; that is to say, the *mode* is 4.8. The mode for all the Caribou loams, typical or not, is 5.0 and is not so sharply defined as the mode for the typical Caribou loam. The mode for the Washburn loam is 6.4 and is still less sharply defined. It is probably more than a coincidence that the Washburn loam is found in the field to be more variable, even over small areas, than the Caribou. Two samples of Washburn loam overlapped the typical Caribou region; as noted previously, these two showed little or no scab. The frequency diagram for all the soils examined (upper diagram, taking into account all the blocks irrespective of the shading) shows two modes; that representing the Washburn loam, however, is not very marked. It will be recalled that in collecting the samples intermediate and atypical specimens (except shale loams) were especially sought. The Caribou shale loam is naturally less acid than the Caribou loam, since here the limestone shale which underlies the soils of this region comes near the surface and is mixed with the surface soil. An explanation for the difference of reaction between Caribou and Washburn loam cannot as yet be offered.

The soils of this region are never limed, for fear of the potato scab, and the Washburn and Caribou loams present a possibility for differentiation of type by hydrogen-ion measurements which is not afforded by limed soils.

The reaction of most of the samples of Washburn loam was suitable for the growth of the potato scab organism. We are in general agreement with the statement of Westover and Rowe (22), that potatoes grown on this soil

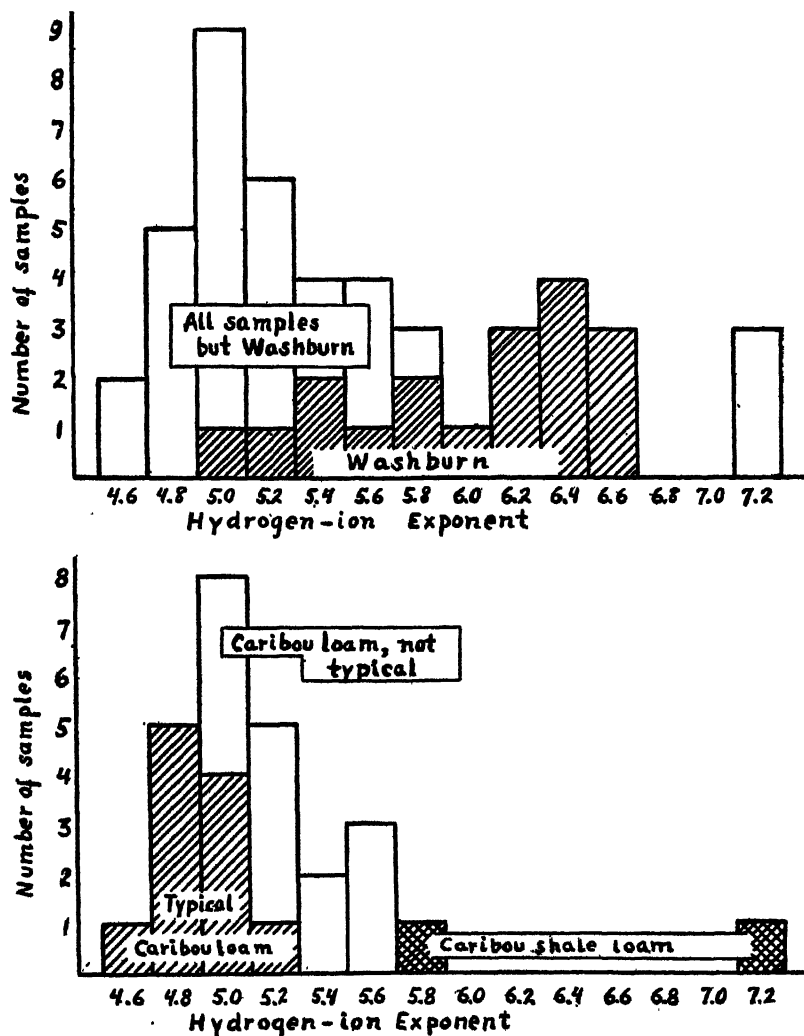


FIG. 3. FREQUENCY DIAGRAMS SHOWING THE NUMBER OF SAMPLES HAVING ANY GIVEN HYDROGEN-ION EXPONENT

type "are inferior and usually scabby, so that it is not advisable to grow them on this soil," even though in a few cases clean potatoes were found and the hydrogen-ion concentration was such as to suggest that in these cases further crops of clean potatoes may be expected.

## ACID-LAND AGRICULTURE

In a very interesting bulletin Coville (5) discussed the relative sensitivity of various crops to soil acidity. He showed that soil acidity is not always an objectionable condition which invariably requires an application of lime, in fact that soil acidity may be beneficial in controlling plant diseases or in preventing malnutrition, and that under certain economic conditions a complete system of acid-land agriculture, based on the selection of acid-tolerant crops in the rotation, is practical and desirable. He concluded that the extent to which our cheap eastern acid lands can be utilized with small applications of lime, or under some conditions without its use, is an important subject for detailed investigation, from which may reasonably be expected results of far-reaching economic importance. He had in mind for the most part soils on which red clover will not grow satisfactorily, but it is now clear that acid-land agriculture is a question of degree, and that an acid-land agriculture is actually being practised in northern Maine, especially on the Caribou loam, upon which potatoes and various truck crops are successfully raised. Liming is not practised. Clover is grown on both Caribou and Washburn loams, a mixture of red clover and alsike generally being sown. In general, red clover does somewhat better on the Washburn loam than on the more intensely acid Caribou loam, but fairly good stands of clover are obtained on the Caribou loam.<sup>6</sup>

Experience with litmus paper has sometimes led to the opinion that a degree of acidity tolerable on one soil type may be excessive on a different soil type; it has been shown, however, that litmus paper can not be relied upon for distinctions of acid intensity in soils (10). There seems to be no reason to suspect that we are dealing, in the case of the Caribou loam, with an exceptional condition in which the degree of acidity represented by the exponent 5 is less harmful than in other soils; on the contrary, other soils having the exponent 5 have come to our attention which do not require liming to produce good yields of potatoes and various truck crops and which include mucks and sands from Florida and some specimens of Norfolk sand.

Because of these facts it is difficult for us to agree with Sharp and Hoagland (21) that "the rational treatment of acid soils requires that sufficient lime be added to bring the soil to a neutral or slightly alkaline reaction." They were taking the exponent 7 as the index of neutrality. In the absence of sufficient evidence, to adopt the exponent 7 as the end-point to be reached in liming would seem quite as arbitrary as to adopt the end-point indicated by any one of several lime-requirement methods, because the exponent 7, though it denotes neutrality in the physico-chemical sense, has no other special

<sup>6</sup> This season (summer of 1918) red clover is doing very poorly in many cases on the Caribou loam; the great difference between the growth of red clover on the two types can almost be used safely for distinguishing the types in the field.

significance.<sup>7</sup> It is quite possible that the exponent 5 may be a sufficient approach to neutrality for many soils and systems of cropping, *whether this intensity of acidity belongs naturally to the soil or is reached by partial liming*. We do not propose the exponent 5 as a standard end-point; on the contrary, we wish to urge that the need is for observation and experiment, not for convention.

#### SUMMARY

Satisfactory procedures for the determination of hydrogen-ion concentration in soils colorimetrically and electrometrically have been described. It is necessary to add one or two cubic centimeters of water per gram of air-dry soil, but this addition of water does not seem to be a serious limitation.

The electrometric and colorimetric results on a large number of soils agreed within the experimental error.

Examination of a large number of soils from northern Maine showed an excellent correlation between hydrogen-ion concentration and occurrence of common potato scab. Soils having a hydrogen-ion exponent as low as 5.2 rarely produced scabby potatoes, soils having exponents much higher generally did produce scabby potatoes. Similar results were found for a few soils of different origin and type. The limiting zone of hydrogen-ion exponent for the potato scab organism appears to be about the same for the soil as had previously been found for culture media.

The characteristic difference of hydrogen-ion exponent between the Caribou and the Washburn loams has been confirmed. The typical Caribou loam has a hydrogen-ion exponent of about 4.8 and is free from scab, whereas the Washburn loam is generally less intensely acid (shows larger exponents) and potatoes grown on it are usually scabby.

A considerable number of soils having the exponent 5 are successfully cultivated in potatoes and truck crops without liming, showing that the exponent 7 (which indicates physico-chemical neutrality) can hardly be taken in general as "the rational" end-point in lime-requirement tests. No such standard end-point is suggested, this being left for future determination with specific crops.

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<sup>7</sup> For instance, it emphatically does not mean that the freely-soluble acid substances in the soil are completely neutralized, though this might be inferred from a recent discussion (13, p. 139-140), or that no more hydrogen ions are present (we do not understand the force of the expression "free hydrogen ions," which is sometimes used), or that conditions are optimal for the growth of acid-sensitive microorganisms.

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# THE SOLUBILITY OF THE SOIL POTASH IN VARIOUS SALT SOLUTIONS<sup>1</sup>

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## HISTORICAL

In this the day of extremely high-priced potash when the supply of potash does not keep up with the demand, the question of whether other elements can unlock potash from the insoluble minerals of the soil is of vital importance. If there are minerals which can liberate soluble potash from some of the insoluble minerals of the soil and thus make available for the use of the plant the immense stores of mineral potash which the soil contains, they should be commended for the use of the farmers.

The various text-books contain many conflicting statements in regard to the power of sodium, calcium and magnesium to replace potassium. It is the purpose of this work to clear up this point, if possible.

The up-to-date publications on soil fertility were examined and it was found that the following said that lime replaced some potash in the soil: Aikman, Blair, Halligan, Hall, Hart and Tottingham, Ingle, Lincoln and Walton, Van Slyke, Vivian, and Voorhees. Briggs and Breazeale, Gaither and Keitt and King and Curry and Smith were dubious as to whether any replacement took place.

The authors who were of the opinion that gypsum replaced potash are listed as follows: Snyder, Hilgard, Aikman, Hopkins, Keitt and King, Ingle, Voorhees and Van Slyke. Harter, Curry and Smith and Murray do not seem to have much faith in the power of calcium sulfate to set free potash.

There is more disagreement among the writers in regard to the action of common salt than as to the action of the calcium compounds. Aikman, Curry and Smith, Snyder, Hall, Storer, Lyon, Fippin and Buckman, and Van Slyke credit common salt with the power of replacing potash in soils. Hart and Tottingham, Voorhees, Murray, and Roberts are doubtful as to the power of salt in this regard.

The question of the liberation of potash is not a new one. E. Wolff [cited by Storer (21)] about 1850 was probably the first to point out the possibility that potash might be replaced by the soda of common salt. He grew a field of buckwheat, one half of which he manured heavily with common salt while

<sup>1</sup> A thesis submitted to the faculty of the Graduate School of Cornell University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

the other half was unmanured. On analyzing the ashes of the buckwheat straw he found that the portion of the crop that had received the salt contained less soda but more potash than the other. (The statement that Wolff was the first to point out the possibility of the replacement of potash by soda in soil, does not infer that Wolff was the first to use common salt as a manure. At that time salt was quite a common manure in England and France, but its value was somewhat debated. In doing his work Wolff was working on the theory of mineral nutrition of plants.)

Boussingault in about 1860 showed the effect of lime and gypsum on the clover grown on some soils, by analyzing the ash of limed and unlimed clover and also plastered and unplastered clover. His results are given in the following tables [cited by Halligan (13)]:

*Composition of clover ash*

	KILOS PER HECTARE			
	Unlimed		Limed	
	First year	Second year	First year	Second year
Lime.....	32.2	32.2	79.4	102.8
Potash.....	26.7	28.6	95.6	97.2
Phosphoric acid.....	11.0	7.0	24.2	22.9

*Composition of clover ash-gypsum tests*

	WITHOUT GYPSUM	WITH GYPSUM
	<i>per cent</i>	<i>per cent</i>
Potash.....	23.6	35.4
Soda.....	1.2	0.9
Magnesia.....	7.6	6.7
Lime.....	28.5	29.4
Oxides of iron and manganese.....	1.2	1.0
Chlorine.....	4.1	3.8
Phosphoric acid.....	9.7	9.0
Sulfuric acid.....	3.9	3.4
Silica.....	20.0	10.4

In the first table it is seen that the percentage of potash in the ash increases more than the percentage of lime. It hardly need be pointed out that the increase of the percentage of potash in the ash might be due to other factors than the liberation or replacement of potash by the lime or the gypsum.

Considerable work was accomplished on the solubility of the soil potash in various salt solutions about the year 1860. Dietrich (7) performed some experiments concerning the solubility of soil constituents in various solutions. He found that the alkali metals of the soil were much more soluble in water containing carbon dioxide than in pure water. He found that calcium car-

bonate dissolved in carbonic acid solutions dissolved about the same amount of total alkalis as did carbonic acid of equal strength. In later work (8) Dietrich found that N/20 solutions of sodium chloride, calcium chloride and lime dissolved much more potassium from soils than did pure water. He found that sodium nitrate and sodium carbonate solutions did not dissolve potash. He concludes that by the manuring of a field with common salt, important amounts of potash are set free.

Eichhorn (9) in 1858 tried the solvent powers of sodium chloride solutions and found that somewhat more potash was dissolved in these solutions than in water. Beyer (2) tried the action of various solutions on feldspars and measured the amount of potash dissolved. Calcium sulfate was not found to have any marked effect on the potash. Sodium chloride solutions and also the nitrate solutions were found to exert a powerful solvent effect on the potash. Sodium nitrate was less active than the chloride.

Harry Snyder (19) in 1893 came to the following conclusion as a result of his experiments on the soils of Minnesota.

The indirect action of land plaster (gypsum) on these soils (western and central prairie soils—black soils resting on yellow clay) in liberating plant-food, particularly potash and phosphoric acid, is unusually marked. Experiments conducted in this laboratory have shown that small amounts of gypsum are quite active in rendering potash, phosphoric acid and even nitrogen soluble in the soil water. It is not the land plaster itself that furnishes the food, but it is the power that it possesses in making mineral matters available that are already in the soil. Land plaster acts more as a stimulant and not as a direct fertilizer, and if not used to excess it will be a profitable fertilizer to use on these soils, especially to bring in grass and clover.

The writer has been unable to find any further reference to the laboratory work which Snyder says was performed at the Minnesota Agricultural Experiment Station.

The Rothamsted Experiment Station has been carrying on experiments concerning the manuring of mangels with soda since 1876. The results (12) seem to indicate that manuring with common salt increases somewhat the percentage of potash in the mangels, but it also increases greatly the percentage of soda in the mangels. The yield of the crop was greatly increased by the addition of soda. The total amount of potash in the crop of mangels was in most cases increased by about one-half. Hall, in discussing these experiments, states that this increase is due to the attack of the soda salt upon the insoluble potash of the soil. The conclusion which Hall (11) draws is quoted as follows:

Since soluble alkaline salts are beneficial to the mangel crop either as direct foods or as economizers of potash, a dressing of salt should always be included among the manures for the mangel crop.

Wheeler (23) in drawing conclusions from the experiments (24, 25, 26, 27, 28, 29) with sodium salts at the Rhode Island Experiment Station, says:



As a result it appeared that possibly unable to wholly replace potassium in any one function, or at least in all of its functions, in connection with the growth of certain plants. sodium may and often does perform some part of one or more of the important functions of potassium and thus increase the amount of dry matter which the plant can produce.

The experiments referred to have been over a period of more than twenty years. Wheeler thinks that the most important function of the sodium salts is not, therefore, the action on the potash-bearing minerals of the soil, but rather as a direct plant nutrient.

Curry, Smith and others (6) of the New Hampshire Agricultural Experiment Station have carried out a large number of experiments relative to the solubility of potash in various salt solutions. They percolated solutions of lime, sodium chloride, sodium carbonate, sodium nitrate, acid phosphate and other salts through columns of soil. They stirred feldspar with these various solutions and also stirred a mixture of feldspar and clay with the solutions. They tried the solubility of feldspar in solutions of lime, gypsum, sodium nitrate, ammonium sulfate, sodium carbonate and disodium phosphate. They found that all of these salts increased the solubility of the potash contained in the feldspar, lime having the greatest solvent action. However, when the feldspar was mixed with clay the lime solution had even less solvent action than pure water. They observed that when solutions of sodium nitrate, sodium chloride, sodium carbonate and acid phosphate are percolated through columns of soil more potash is made soluble than when water is percolated. They conclude that calcium carbonate and lime have practically no effect on the solubility of the soil potassium, and that the calcium sulfate makes but small amounts of potassium soluble. However, they say that the effect of sodium chloride, sodium nitrate, sodium carbonate and acid phosphate is to greatly increase the solubility of the soil potassium. The reaction, they believe, between these salts and the soil is chemical.

Soderbaum (20), working on plot experiments in 1911, however, concluded that the beneficial results obtained from the use of common salt as a fertilizer were due to the effect of the chlorine which was introduced and not to the sodium.

Bradley (3) of the Oregon State Agricultural College worked on the effect of lime and gypsum upon the soils of Oregon. He mixed the soils with either lime or gypsum in large glass percolators and allowed the soils to stand at the optimum moisture content for six weeks. He analyzed the solutions obtained by leaching these soils. He found that both lime and gypsum set potash free. The gypsum, however, was more active in this regard than the lime. He also tried shaking the soils with solutions of lime and gypsum. In this case he found that the lime decreased the amount of potash going into solution, whereas the gypsum increased it greatly. He concludes that gypsum sets free potash in the soils of the Willamette Valley.

Gaither, of the Ohio Agricultural Experiment Station (10) working with plot tests determined the solubility of the various elements in N/5 nitric acid.

He concludes that lime breaks up certain silicates in the soil and renders them more soluble in N/5 nitric acid but does not act upon the insoluble potassium compounds in the soil to such an extent that N/5 nitric acid can be used as a measure of such potash. The addition of caustic lime has the effect of diminishing the amount of potash assimilated by wheat grown on such soil. The theory that lime added to the soil increases the amount of available potash in the soil is either erroneous or requires more positive proof than has heretofore been obtained, before it can be accepted.

Andre (1) in 1912 worked on the replacement of potash in certain feldspathic rocks by the addition of sea salt or of sodium nitrate. He found that the potash of microcline was quite noticeably dissolved by solutions of sea salt or sodium nitrate; the amount of potash going into solution being almost the same in both cases. He concludes that the replacement explains the favorable action of salt when used as a fertilizer. He thinks that sodium nitrate is valuable as a fertilizer not only for the nitrogen that it furnishes, but also because the sodium added to the soil sets free a certain amount of potash.

Iakushkin (16) found that the addition of sodium chloride as a fertilizer increased the yield of Japanese millet 52 per cent. The beneficial effect of sodium was observed in a complete normal nutrient solution, thus indicating that the action of sodium is not due to the replacement of potash.

Hartwell and Wessels have published data (14) concerning the experiments at the Rhode Island Agricultural Experiment Station of a more recent date than that of Wheeler previously referred to. They observe that soda can partially replace potassium as a fertilizer for mangels and onions. If liberal applications of sodium manures were applied, an equally large yield of onions and mangels were obtained even when the amount of potash manures had been reduced one-third. However, when the potash ration was reduced one-half, in some cases the crop yield was reduced somewhat.

Briggs and Breazeale (4) of the Bureau of Plant Industry, have recently finished some work which seems to prove the opposite of much of the work which has been cited. Their article in the *Journal of Agricultural Research* excited considerable comment and a number of persons, including the writer, have attempted to duplicate the results which they reported. They determined the solubility of pegmatite and orthoclase in calcium hydroxide and calcium sulfate solutions of various concentrations. The calcium hydrate solutions did not modify the solubility of the potassium in either pegmatite or orthoclase. Gypsum solutions depressed the solubility of the potassium in orthoclase, the quantity of potash in the solution decreasing progressively as the concentration of the calcium sulfate solution increased.

Similar tests were made upon a virgin soil of a granitic type. The solubility of the potash was not measurably different in distilled water and in solutions of calcium sulfate or calcium hydroxide. In the case of a soil of similar nature, which had been under cultivation for some time, which was somewhat more

granular and less weathered than the virgin soil, the addition of calcium sulfate decreased the solubility of the potash. They conclude:

The experiments indicate that the availability to plants of the potash in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum. In some instances a marked depression of the solubility of the potash in the presence of gypsum was noted.

In some recently published work (17) concerning some lysimeter experiments, Lyon and Bizzell have shown that the application of lime to soils did not result in an increase in the quantity of potash contained in the drainage water, nor in any increase in the amount of the potassium contained in the crops.

From the foregoing review of the literature, it is seen that about the year 1860 it was generally accepted that calcium and sodium salts did liberate potash from the soil. Although this was accepted as a fact, insufficient proof was given. Recently Wheeler has suggested that perhaps the plant did not derive its benefit from the potash set free when salt was applied to the soil, but from the element itself. Within the last decade a number of experimenters have again attacked the problem of liberation of potash. Different men have obtained different results. There is not the agreement of results from which the truth can be deduced. The writer wishes to point out that the various workers have used different types of soil and that it is but natural that they should get different results. No general statements can be made from the experimental work performed with one type of soil. Experimenters should use many types of soil from many localities and then draw their conclusions for the types of soil used in their experiments.

#### EXPERIMENTAL

In this work no effort was made to determine the nature of the phenomenon of the liberation of potash from the soil minerals by the salt solutions. The writer determined the amount of potash that dissolved in salt solutions. An attempt was made to determine the effect of the concentration of the solution. Various types of soils were studied with particular reference to the effect of solutions of calcium sulfate upon the potash which they contain. Besides studying the effect of the various calcium salts upon the soil potash, the effects of various sodium salts were studied.

The solubility of the soil potash in carbonic acid and in calcium bicarbonate was compared, in order to throw some light upon the action of lime or calcium carbonate upon the potash contained in the soil minerals.

Soils which had been manured with gypsum for years were compared in regard to the solubility of the soil potash in gypsum solutions with check soils, which had never been treated with gypsum. It was hoped that this might give some information on the residual effect of gypsum as a fertilizer.

## THE METHOD

The method used in the experiments was practically the same in all cases. It consisted in merely allowing the soil to remain in contact with the various salt solutions until the systems came to equilibrium. One hundred and twenty five grams of dry soil, or its equivalent in moist soil, was placed in a liter of water contained in a 2-liter bottle. Various amounts of salts were added to the solutions and they were allowed to stand for 3 weeks. During this period they were shaken once or twice a day. In order to determine whether the soil potash was more or less soluble in the salt solutions, blank determinations were run in all cases. In other words, no salts were added to some of the soil samples but the soil was merely allowed to stand in contact with the water under the identical conditions.

In case this procedure was altered in any way, it is noted in connection with the results.

It was determined by experiment that at least 2 weeks are required for the soil to come into equilibrium with the solution. At the end of a week the amount of potash found in one of the solutions was 2.1 parts per million. At the end of 2 weeks the same solution contained 3.2 parts of potash per million and at the end of 3 weeks the amount had not changed perceptibly. In order to be reasonably sure that the soil was in equilibrium or nearly so with the solution, the solutions were allowed to remain in contact with the soil for 3 weeks. At the end of that time the solutions were decanted off from the soil and were filtered through a Pasteur-Chamberland porcelain filter.

Potash was determined in the solutions. The method used was a modified Cameron and Failyer method. This method was first described by the above men in the *Journal of the American Chemical Society* (5). To an aliquot of the solution some ammonium oxalate solution and a couple drops of a 10 per cent ammonium carbonate solution were added. The amount of ammonium oxalate added depended upon the amount of calcium in the solution. If no calcium salt was added only three or four drops of a saturated solution of ammonium oxalate was added. The solution was then heated to boiling and boiled for a minute and then filtered. The filtrate was collected in an evaporating dish and the solution evaporated to dryness on a water bath. Enough dilute sulfuric acid was then added to moisten the salts and the dish was then heated at a dull red heat until the ammonia was completely driven off.

The salts were then taken up in a little water, a drop of pure concentrated hydrochloric acid was added, and then sufficient 0.25 per cent chloroplatinic acid to react with all the sodium and potassium salts present, after which the solution was evaporated to a paste on a water bath. The paste was then taken up in alcohol. Ninety-five per cent alcohol was used, for the potassium chloroplatinate is less soluble in alcohol of that strength than it is in 80 per cent alcohol. The solution was then filtered through asbestos into a Gooch

crucible. The potassium chloroplatinate was well washed with alcohol. When no large amount of sodium was present 100 cc. of alcohol were used. But when sodium salts were added to the solution it was found necessary to wash the chloroplatinate with 150 cc. of alcohol in order to dissolve all of the sodium chloroplatinate. After drying the crucible in an oven at 100° for a half-hour, the potassium chloroplatinate was dissolved in about 50 cc. of hot water and a drop of concentrated hydrochloric acid was added. After cooling, a solution of potassium iodide containing at least ten times more than enough potassium iodide to react with the chloroplatinate was added and the solution allowed to stand over night. During this time a rose color developed in the solution and the next morning the color was compared in a colorimeter with a standard solution containing a known amount of potassium chloroplatinate prepared in a similar manner. The most trouble was experienced in obtaining checks when sodium salts were present in large quantity.

When no sodium had been added to the solution no trouble was found in getting the determinations to check within 0.5 part per million. When large amounts of sodium were present checks were usually within one part per million.

#### DESCRIPTION OF SOILS

The soil called Dunkirk silt loam which was used in many of the experiments is a light brown silt loam. It belongs to the "Glacial lake and river terrace province." It is underlain by a slightly heavier subsoil of a brown color. It is of a sedimentary origin and represents the wash from the higher slopes deposited in quiet glacial lake waters. It is a good soil for general crops. That used in the experiments contained 4.7 per cent of organic matter (determined as loss on ignition) and 1.9 per cent of potash. It was slightly acid to litmus paper. It was obtained near Ithaca, New York.

The soil called Whiteland clay subsoil was obtained in the town of Corvallis, Oregon. It belongs to the second bench Willamette type. It is of sedimentary origin and was laid down by the Willamette River. Whiteland is a poorly drained soil of mottled gray and brown color. It is underlain by a heavy gray clay and this is the soil used in the experiments. It contained 6.2 per cent of organic matter (determined as loss on ignition) and 1.94 per cent of potash. It reacted neutral to litmus. This is a very poor soil for all crops.

The Yamhill silt loam was obtained from near Corvallis, Oregon, and is an alluvial soil laid down by the Willamette River. It belongs to the first bench soils of the Willamette series. It is a brown loam soil with a brown subsoil of finer texture than the surface soil. It reacted neutral to litmus. The sample analyzed contained 1.5 per cent of potash and lost 4.8 per cent on ignition. It is a very fertile soil.

The Porters sandy loam belongs to the "Appalachian soil province" and is of residual origin. The sample used in these experiments was obtained from North Carolina. It is of igneous rock origin, occupies mountainous land,

and is dark gray in color. The sample used contained many small glistening scales of mica. It contained 10.2 per cent of organic matter; its potash content was 1.2 per cent, and its reaction towards litmus was neutral. The sample was from a fertile field. Wheat, corn, oats, rye and potatoes are the principal crops grown on it.

The Durham sandy loam is a soil belonging to the Piedmont soil province. The sample used in the experiments was obtained from North Carolina. The soil is a light sandy loam underlain by a pale yellow sand. The type is derived from a light-colored granite. Owing to a lack of organic matters the soil dries out quickly. The sample taken, however, contained more than the average amount of organic matter for this type—8.7 per cent. Its potash content was 0.4 per cent. It reacted slightly acid to litmus. The soil is not especially fertile.

The Genessee humus loam is a recently formed alluvial soil, formed from reworked glacial till. The sample used was obtained near Ithaca, New York. It was mottled black and brown and although it contained considerable coarse sand it also contained a relatively high proportion of clay. It was of only average fertility, although it contained 15.7 per cent of organic matter. Its reaction towards litmus was neutral.

The Merrimac fine sandy loam was obtained from the experimental plots of the Massachusetts Experiment Station, Amherst, Massachusetts. The surface soil consists of a light-brown fine sandy loam. This type occurs as narrow terraces along rivers and represents glacial flood-plain deposits. The soil is a very fertile one, and brings a high price per acre, onions and tobacco doing particularly well on it. It contains 2.2 per cent of organic matter and 1.7 per cent of potash.

The chief idea in choosing the above soils was to get a variety of soils of different types and from various sections of the United States. General deductions cannot be drawn by merely experimenting with one type of soil from only one locality.

#### EXPERIMENTS CONCERNING THE SOLUBILITY OF THE SOIL POTASH IN ACID PHOSPHATE SOLUTIONS

Curry and Smith (6) of the New Hampshire Agricultural Experiment Station, experimented with an acid phosphate free from potash and determined the solubility of the soil potash in a very dilute solution of this phosphate. They percolated this dilute solution through a column of soil and found that there was a considerable increase in the amount of potash in the percolate. They concluded that the effect of commercial acid phosphate when applied as a fertilizer is to greatly increase the solubility of the soil potassium. They however did not attribute the action of the acid phosphate to any one compound contained in the acid phosphate.

Commercial acid phosphate consists chiefly of a mixture of monocalcium phosphate, dicalcium phosphate, tricalcium phosphate and calcium sulfate. Any liberation of potash would be due to one or all of these compounds. Dunkirk silt loam was shaken with saturated solutions of these salts and the amount of potash in the solutions noted. The results are given in table 1.

In this and all subsequent tables the amounts of potash are expressed as  $K_2O$  in parts per million of solution.

It is seen from the table that the only substance which increases the solubility of the potash to any appreciable extent is the calcium sulfate. Yet in this same paper in which Curry and Smith state that acid phosphate is so active in liberating potash from the soil, they say that "A limited number of experiments with calcium sulfate indicate that *small* amounts of potassium are made soluble."

The results of the above experiments seem to indicate that tricalcium phosphate has little or no action on the soil potash. The same is true in regard

TABLE 1  
*Potash liberated in Dunkirk silt loam*

SALT	CONCENTRATION OF POTASH IN SOLUTION IN PARTS PER MILLION	
	A	B
Merely distilled water.....	3.8	3.4
Calcium sulfate.....	11.8	11.8
Tricalcium phosphate.....	3.2	3.0
Dicalcium phosphate.....	3.4	4.0
Monocalcium phosphate.....	1.5	2.0

A and B are duplicate experiments.

to the dicalcium phosphate while the monocalcium phosphate seems to have a negative effect. It seems therefore that if acid phosphate has any beneficial effect on soil in making potash soluble, this effect is due to the calcium sulfate which it contains.

In order to learn whether the above conclusion held for other soils, two other soils were tried with the same salt solutions. In this experiment, a Whiteland clay subsoil and a Yamhill soil were tried. The results were similar, except in the case of the calcium sulfate solution. They are listed in table 2.

In these other soils the calcium sulfate does not seem to have any action in making potash soluble. In one case, the dicalcium phosphate and tricalcium phosphate seem to keep the potash from going into solution. In the case of the Yamhill soil which is coarser in texture than the other soils, these phosphates have little action on the potash. In all three soils the monocalcium phosphate apparently hinders the solution of the potash. We must conclude,

therefore, that only on certain types of soil does calcium sulfate have any action. And since calcium sulfate is the active principle of commercial acid phosphate, the same holds true of acid phosphate.

TABLE 2  
*Potash liberated in Whiteland clay subsoil and Yamhill soil*

SALT	CONCENTRATION OF POTASH IN SOLUTION IN PARTS PER MILLION	
	Whiteland subsoil	Yamhill soil
Distilled water only.....	3.2	3.0
Calcium sulfate.....	3.2	3.2
Tricalcium phosphate.....	1.8	3.2
Monocalcium phosphate.....	0.9	0.9

#### EXPERIMENTS CONCERNING THE SOLUBILITY OF THE SOIL POTASH IN SOLUTIONS OF CALCIUM SULFATE

The above-outlined experiments indicated that all soils were not uniformly affected by calcium sulfate solutions. Seven soils, from four different states, varying widely in characteristics and properties, were taken and tested as to their solubility in saturated calcium sulfate solutions. The results are listed in table 3.

TABLE 3  
*Results showing solubility of soil potash in solutions of calcium sulfate*

SOIL	Distilled water	Calcium sulfate solution
	p. p. m.	p. p. m.
Whiteland clay subsoil.....	3.2	3.2
Dunkirk silt loam.....	3.5	11.8
Yamhill silt loam.....	3.0	3.2
Genessee humus loam.....	5.5	7.4
Merrimac fine sandy loam.....	1.0	1.3
Durham sandy loam.....	4.8	5.7
Porters sandy loam.....	3.5	3.7

Aside from the Dunkirk silt loam, Durham sandy loam and Genessee humus loam are the only soils that showed any marked effect of the action of the gypsum solution.

Because of the extraordinary action of solutions of calcium sulfate upon the Dunkirk silt loam the writer experimented further with this soil. Five hundred grams of it were taken and separated mechanically into clay, silt and sand. The sand was considered as being composed of those particles



above 0.05 mm. in diameter; the silt, those particles between 0.05 and 0.005 mm. in diameter; and the clay all particles of smaller diameter than 0.005 mm. The soil was shaken for 12 hours to deflocculate it. The clay and silt were then separated from the sands by simple subsidence and decantation. The silt was separated from the clay by whirling the soil in the water in tubes in a centrifuge. After the separation the soil separates were dried. Thirty grams of the three separates were treated with 500 cc. of an approximately saturated calcium sulfate solution for 3 weeks. Like amounts of the separates were treated with a like amount of distilled water. At the end of 3 weeks the soil solutions were filtered and the potash determined in the solutions. The results are presented in table 4.

The sand contained but 2.2 per cent of organic matter while the silt contained practically the same amount as did the entire soil, 4.6 per cent. There was not enough of the clay left over to determine the organic matter (as loss on ignition) but the clay must have been high in organic matter, as that must have contained the organic matter which was missing from the sand. Some organic matter was probably dissolved and this would be contained in the clay.

TABLE 4

*Results showing the solubility in calcium sulfate of potash in Dunkirk silt loam*

SOLUTION	SEPARATE			
	Entire soil	Sand	Silt	Clay
Distilled water.....	4.7	3.2	5.9	17.9
Calcium sulfate.....	8.1	4.7	8.8	25.1

It is noteworthy that although some liberation of potash occurs in all three separates, the greater portion of the liberation occurs in the clay. As the particles become smaller the solubility of the potash increases and the amount made soluble by the gypsum increases.

In the above experiment the amount of potash dissolved by water alone was higher than in the previous experiments. This experiment was carried out in hot weather, whereas the previous experiments were conducted during cold weather. This was one of the factors which caused the increased solubility of the soil in distilled water.

Way (22) in 1850 found that soil absorbed potash. Solutions of potassium nitrate were filtered through columns of soil and the percolate contained no potash. In the above experiments the soil was treated in a fine suspension in solution and was well shaken so that the clay, which possesses most of the absorbing power of the soil, was entirely in suspension. It is conceivable that when a solution of calcium sulfate slowly percolates through the soil, as is the case when gypsum is added to the soil as an amendment, the gypsum may liberate the potash and have it remain in the soil. In fact, it would be sur-

prising if we would have any very large amount of potash in the drainage water from any lysimeter containing a heavy clay soil. The fact, then, that the analysis of water from lysimeter tanks does not show an increased amount of potash in solution due to the addition of gypsum to the soil does not necessarily prove that there is no liberation of potash by the gypsum. Experiments such as the above, in the writer's opinion, would be more positive proof that potash is made more soluble. For if the potash were liberated there would be little chance for the clay to absorb it and remove it from solution.

Insufficient work has been done to make it possible to state definitely in just what soils a marked replacement of potash could be expected. The Porters sandy loam was of average potash content (1.2 per cent) and even contained a large number of flakes of mica, yet there was practically no replacement of potash. Curry and Smith and others have found that gypsum solutions will dissolve some potash from the natural potash-bearing rocks, but the amount is small. The writer is of the opinion that the action on the clay is much more important. It seems probable that there will be an increase in the solubility of the potash due to calcium sulfate in fertile clays and loams containing a considerable amount of potash.

TABLE 5

*Results showing liberation of potash from Dunkirk silt loam by different concentrations of calcium sulfate*

	CONCENTRATION OF SOLUTIONS				
	No calcium sulfate	1.36 gm. per liter	0.68 gm. per liter	0.34 gm. per liter	0.17 gm. per liter
	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.
A	5.1	8.9	8.9	8.4	7.0
B	5.2	8.9	8.8	8.1	6.8

A and B are duplicate experiments.

In order to determine how much calcium sulfate must be present in solution in order to have any appreciable liberation of potash from Dunkirk silt loam, solutions of different concentrations were used and the amount of potash dissolved measured as before. The results are given in table 5.

Figure 1 shows graphically the amount of potash set free at any concentration of solution. It should be noted that 1.36 gm. of calcium sulfate is a third more than will dissolve in a liter of water. This partially explains why half as much of the salt set free as much potash.

The fact that 0.170 gm. of calcium sulfate in a liter of solution, or, in other words, 170 parts per million, made 1.75 parts per million of potash soluble is surprising. If 150 pounds of calcium sulfate were applied to an acre of soil and if the gypsum all dissolved in the moisture in the first foot, at a 20 per cent moisture content the concentration of the calcium sulfate would be approximately 170 parts per million. This may account for the fact that on

some soils, merely small applications of calcium sulfate have a remarkable action in increasing the crop yields. When large amounts of gypsum are applied to soils the deleterious physical effect of the addition seems to overcome the benefits derived from its use.

Through the kindness of Dr. F. W. Morse, acting-director of the Massachusetts Agricultural Experiment Station, samples of soil were obtained from the plaster and check experimental plots of that station. The soil is of the Merrimac fine sandy loam type, and is considered a very fertile soil. Soil from plot 11 and plot 12, which is the check plot, was obtained. Plot 11 has

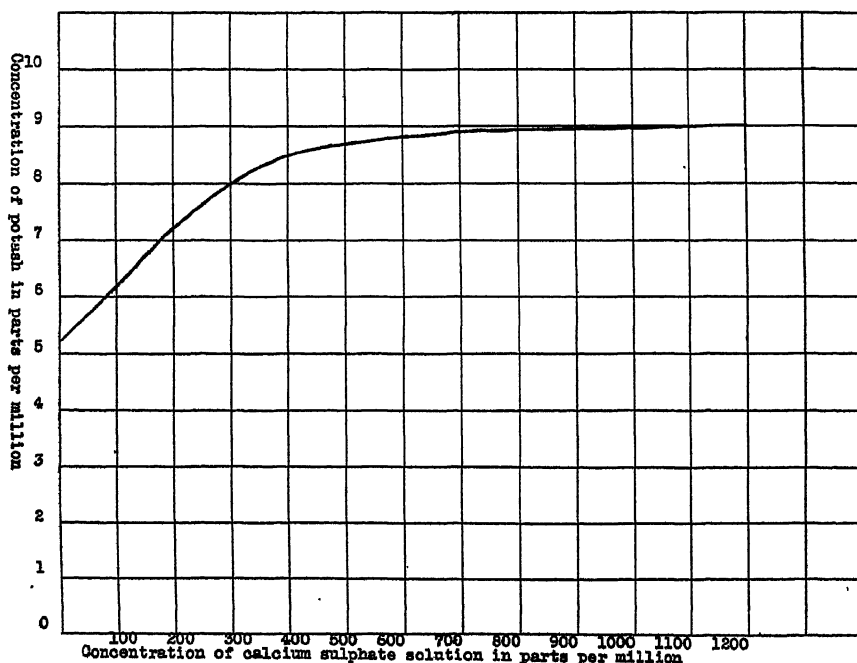


FIG. 1. CURVE SHOWING EFFECT OF CONCENTRATION OF CALCIUM SULFATE SOLUTION ON THE SOLUBILITY OF SOIL POTASH

been dressed annually with 800 pounds of "plaster" since 1890. These soils were tested with calcium sulfate solution in the same manner as the other soils, except that the soils remained in contact with the solutions for 5 days only. However, they were shaken more frequently. The results of these experiments are shown below.

As in the following experiments, the calcium sulfate solution used was a saturated solution.

No very definite facts can be deduced from the results of the above experiment, but it seems as if the potash in a soil which has been treated with gyp-

SOIL	POTASH DISSOLVING IN WATER		POTASH DISSOLVING IN GYPSUM SOLUTION	
	A	B	A	B
	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>
Plot 11 (plastered).....	1.2	1.3	1.5	1.4
Plot 12 (check).....	1.0	1.0	1.2	1.5

A and B are duplicate experiments.

sum for a large number of years was slightly more soluble than in the untreated soil. This statement requires more conclusive evidence than the above, however, before it can be considered as a fact.

#### EXPERIMENTS CONCERNING THE SOLUBILITY OF THE SOIL POTASH IN SOLUTIONS OF CALCIUM CARBONATE

Inasmuch as calcium oxide and calcium carbonate are annually added as amendments to the soils of the United States in large quantities, it is important that we know whether or not calcium-carbonate solutions have any similar action on the soil potash. Calcium-carbonate solutions alone were tried (calcium carbonate mixed with soil and distilled water) but because of the very slight solubility of the carbonate the results were negative. They are listed below:

SOIL	POTASH DISSOLVING IN WATER	POTASH DISSOLVING IN CALCIUM CARBONATE SOLUTION
	<i>p. p. m.</i>	<i>p. p. m.</i>
Whiteland clay subsoil.....	3.2	2.4
Yamhill silt loam.....	3.0	3.0

In each case, 2 gm. of calcium carbonate were added to a liter of water. In the case of the Whiteland clay subsoil, the presence of the calcium carbonate seemed to depress the amount of potash going into solution. The results of the analyses checked closely, but it is possible that they are in error. At any rate, there could be no increased solubility of the potash.

Inasmuch as the amount of calcium carbonate going into solution in a soil water is controlled almost entirely by the carbon dioxide which the water contains dissolved in it, it seemed logical to try the solvent action of calcium bicarbonate on soil, or in other words, a solution of calcium carbonate in carbon dioxide and water. In these experiments 125 gm. of Dunkirk silt loam were taken. Two grams of calcium carbonate and a liter of water were added to this soil. The whole was shaken up and kept saturated with carbon dioxide for 5 days, after which the bottles were stoppered up and allowed to stand, with the exception of shaking up once a day for 3 weeks. At the end of this

time the solutions were analyzed for potash, as were the other solutions. The results of these analyses are given in table 6.

For purposes of comparison the check determinations with water and with water saturated with carbon dioxide are given in the table. In the case of Dunkirk silt loam N/50 calcium bicarbonate caused an average increase in solubility of 1.55 parts of potash per million. In cases where a ton or more of lime is added to the acre there may be an appreciable amount of calcium carbonate dissolved in the soil water. In such cases some potash may be affected by the calcium bicarbonate.

TABLE 6

*Results showing amounts of potash liberated from Dunkirk silt loam by calcium carbonate solutions*

	SOLUTION		
	Distilled water	Water saturated with carbon dioxide	Calcium bicarbonate
	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>
A	5.3	8.4	9.6
B	5.2	8.7	10.8
Average.....	5.25	8.55	10.1

A, and B are duplicate experiments on the same soil.

#### EXPERIMENTS ON THE SOLUBILITY OF SOIL POTASH IN SOLUTIONS OF VARIOUS SODIUM SALTS

In studying the action of solutions of sodium salts on soils, sodium nitrate, sodium carbonate and sodium chloride were first tried. Table 7 shows the effect of these solutions on Whiteland clay subsoil and Yamhill silt loam.

In the experiment 1.7 gm. of sodium nitrate, 1.169 gm. of sodium chloride and 1.06 gm. of sodium carbonate were taken. The amounts mentioned were enough to give one-fiftieth of an atomic weight of sodium in grams in a liter of solution. These figures certainly show that the soil potash is much more soluble in solutions of these salts than in water. The reason why sodium nitrate should be less active, in this regard, than sodium chloride or sodium carbonate is not clear.

In order to ascertain whether or not other soils were affected similarly by sodium salt solutions, some other soils were tested out in the same fashion. For purposes of comparison, the above-reported results are included in table 8. The amount of salt added was the same in both cases.

The potash in all soils is dissolved to a greater or lesser extent by common salt solutions. The amount going into solution is much greater in the case of the sodium salt solutions than in the case of the calcium sulfate solution or of calcium carbonate solutions. It is noteworthy, that, of the soils listed in

TABLE 7

*Results showing the effect of various sodium salts on soil potash*

SOIL	POTASH DISSOLVED BY THE SOLUTION			
	Distilled water	Sodium nitrate	Sodium chloride	Sodium carbonate
	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>
Whiteland clay subsoil.....	3.2	4.9	13.3	14.3
Yamhill silt loam.....	3.0	8.6	22.6	22.9

TABLE 8

*Results showing the effect of sodium chloride on the potash in different soils*

	POTASH DISSOLVED BY THE SOLUTION	
	Distilled water	Sodium chloride solution N/50
	<i>p. p. m.</i>	<i>p. p. m.</i>
Whiteland clay subsoil.....	3.2	13.3
Yamhill silt loam.....	3.0	22.6
Genessee humus loam.....	5.5	13.0
Durham sandy loam.....	4.8	14.8
Porters sandy loam.....	3.5	14.6

TABLE 9

*Results showing the effect of the concentration of the sodium chloride solution on the solubility of potash in Yamhill silt loam*

CONCENTRATION OF THE SODIUM CHLORIDE SOLUTION	POTASH DISSOLVED
<i>grams per liter</i>	<i>p. p. m.</i>
0.0000	1.8
0.0730	1.9
0.1460	4.1
0.2923	12.5
0.5840	15.2
1.1690	22.6

table 8, the Yamhill silt loam is the most fertile, and that it is also most affected by the sodium chloride solution.

In order to determine the effect of the concentration of the salt solution, the amount of potash going into solution was determined in solutions of sodium chloride of various concentrations. In this experiment a different sample of Yamhill silt loam was used. The results are listed in table 9.

Figure 2 shows the results graphically. It will be noted that the increased solubility of the potash does not amount to much until the concentration of the salt solution reaches about 290 parts per million. At lower concentrations the increased solubility of the potash is hardly noticeable. This may

account for its successful use on beets, whereas it is harmful to a number of other crops. Beets and mangolds need a large amount of potash. They seem to require it in the photosynthesis of sugar. According to Shaw (18) beets are not harmed even when the amount of sodium chloride in the first four feet of an acre rises to 10,000 pounds. Hilgard (15) lists the common crops grown on alkali soils, and sugar beets stand third in order of their resistance to sodium chloride, the only crops surpassing it in this respect being

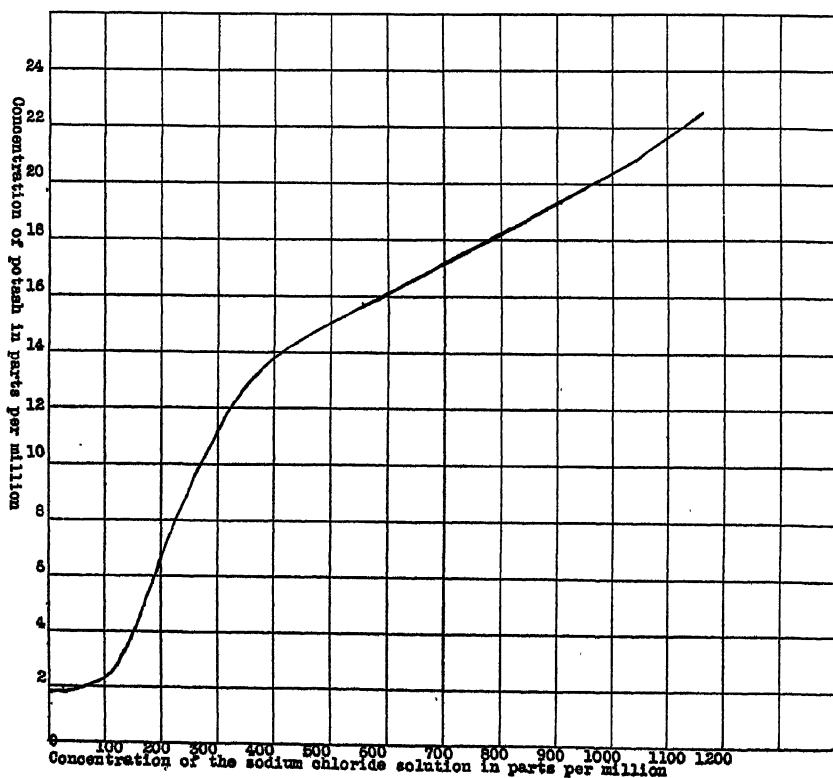


FIG. 2. CURVE SHOWING THE EFFECT OF THE CONCENTRATION OF THE SODIUM CHLORIDE SOLUTION ON THE SOLUBILITY OF SOIL POTASH

salt grass and modiola. Hall (12) advises the use of sodium chloride as a manure for beets, and in England it is a common practice to include sodium chloride in the fertilizers for beets. It may be that the beet, not being harmed by the increased concentration of salt in the soil solution, derives benefit from the potash made soluble by the interaction of the salt with the soil. Such is within the realm of reason, for the concentration of sodium chloride, in the soil solution would only have to rise to about 300 parts per million in order to make considerable potash soluble.

## SUMMARY

Certain soils were tried as to their solubility in solutions of the various components of commercial acid phosphate. It was observed that gypsum did exert a solvent action on the potash compounds of the soil. The solubility of the potash in various soils was determined in calcium sulfate solutions. The solubility of the potash in Dunkirk silt loam was determined in solutions of calcium sulfate of various concentrations.

The solubility of the potash in Dunkirk silt loam in solutions of carbon dioxide and calcium bicarbonate was measured.

The action of various sodium salts in making soil potash soluble was observed. The solvent action of sodium chloride solutions on different soils was measured and the effect of the concentration of the sodium chloride solution on the solubility of the soil potash was tested.

## CONCLUSIONS

1. If commercial acid phosphate has any action in liberating potash in the soils used in the experiments, it is due to the gypsum which it contains.

2. Calcium sulfate in solution does increase the solubility of the potash compounds in some soils. This action is much more marked on the clay than on the silt or sand. This may explain the fact that only some soils are benefited by applications of gypsum. Calcium sulfate solutions do not seem to be particularly active in dissolving the potash of silt and sands containing mica. It is probable that on some, if not all, fertile clay loam and clay soils, some potash is made soluble by the application of gypsum.

3. In the case of Dunkirk clay loam and silt loam, only a small amount of calcium sulfate need be present in the solution in order to affect materially the solubility of the potash. This may explain why small applications of gypsum are quite beneficial on some soils.

4. The soil potash of Dunkirk silt loam is somewhat more soluble in solutions of carbon dioxide and calcium bicarbonate than it is in a solution of carbonic acid containing the same amount of carbon dioxide. Soils which are high in organic matter may derive some soluble potash from the effect of the calcium bicarbonate in the soil water after the addition of a large amount of lime.

5. Sodium salts are quite active in dissolving potash from soils. The fact that sodium chloride solutions are active in dissolving potash may partially explain why beets derive benefit from applications of salt, since beets are very resistant to the toxic action of sodium chloride. Wheeler points out that beets need sodium for proper growth. These two facts taken together may explain the benefits obtained by the use of salt on certain crops.



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# THE PRESENCE OF ALUMINUM AS A REASON FOR THE DIFFERENCE IN THE EFFECT OF SO-CALLED ACID SOIL ON BARLEY AND RYE<sup>1</sup>

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## INTRODUCTION

It has been found by means of field experiments conducted at the Rhode Island station that under conditions where liming exerted very little influence upon the growth of rye, barley was increased two to threefold (4). These crops were selected in the present instance simply as convenient representatives of crops differently affected by acid-soil conditions.

In a previous article by us (5), the method of water culture there described being the same as in the present instance, it was shown that barley seedlings were not more susceptible than rye seedlings to injury by acidified nutrient solutions, even though so-called acid soils are much more deleterious to barley than to rye. The reason for the different effect of acid soil and acid nutrient solutions has interested the authors for a number of years. The present paper is a record of their intermittent work on the subject.

Unless otherwise stated, the solution work with seedlings was conducted in 250 cc. wide-mouthed bottles under such conditions that the transpiration could be measured during the few weeks of the experimental period. The total transpiration could be compared with the final weight of the green tops.

## EFFECT OF ACID IN WATER AND SAND CULTURES

There are so many different cultures involved in the present work that a condensation of the data seems desirable. Therefore, in most cases the transpiration and weight of the green tops accompanying each bottle are not given, but the effect of a stated treatment is considered to be shown by comparing the average of both these observations derived from duplicate cultures. For example, in recording some additional data concerning the relative effect on rye and barley seedlings of sulfuric acid added to the nutrient solution, the depression in growth of the rye based upon the averages of transpiration and weight of the green tops, as just explained, is represented by 100 in each case, and the depression in the growth of barley in individual instances is ex-

<sup>1</sup> Contribution No. 247 from the Agricultural Experiment Station of the Rhode Island State College.

pressed as related thereto. In different experiments and under a number of different conditions the depressing effect on barley caused by the acid was, in comparison with 100 as representing the effect on rye, 124, 103, 102, 101, 95, 100, 99, 99, 96, 121, 98, 98, 96, 120, 75, 113; average, 103.

This agrees with the earlier work in showing that barley and rye seedlings are affected practically alike by acid added to a nutrient solution containing the usual essential elements.

Realizing that conditions attending the growth of seedlings in water culture are quite different from those in the field, a nearer approach to field conditions was made by growing the seedlings in sand and nutrients with and without the addition of acid, usually sulfuric. Sometimes sea-beach sand and again quite pure quartz sand was used without any special treatment. In other experiments the sand was digested in hot acid, washed, and ignited previous to its use. If the depression in the growth of rye is expressed in each case by 10, the relative depression in the growth of barley becomes 18, 8, 23, 11, 22, 9, 13, 6, 9, 10, 10, 13, 10, 13, 14, 4, 12, 17, 25, 18, 26, 10, 12, 15, 21, 27, 16, 8, 17, 10, 10, 23, 12, 6, 13, 4, 12, 13, 14, 13, 10, 15, 13, 11, 16; average, 14.

It may be seen that on the average the sulfuric acid depressed the growth of barley more than it did that of rye. In many instances, however, the difference was slight, as was nearly always the case in water cultures, and the authors were inclined to the opinion at the time that the exceptions were accountable mainly to abnormal differences in the relative vigor of the two kinds of seedlings. For the purpose of determining the difference in the effect of acid on various strains and varieties of barley and rye, a number of samples of these grains were kindly furnished upon request, by the agricultural experiment stations of the states of Utah, Minnesota, Iowa and Pennsylvania, and of the province of Ontario.

It was found by carrying on water cultures with these seeds that when the various strains were grown under the same conditions, either in neutral or acid cultures, there were some marked differences in the relative results. Therefore, in order to ascertain whether the relative effect of acid on the two kinds of seedlings was really dependent on the method of culture, water cultures and prepared sand cultures were carried on at the same time and with the same lot of seeds in each of a number of tests. The results are given in table 1, where it may be seen that the relative depression was about the same with the two kinds of seedlings, both in the water and sand cultures.

These results show that acidity present in connection with the ordinary plant nutrients has a relative effect on the two kinds of seedlings very different from that of so-called acidity in connection with the soil; for, as has been shown in the field repeatedly at this station, barley is much more sensitive than rye to acid-soil conditions.

TABLE 1

*The relative effect of acidity on the weight (in grams) of the green tops of barley (B) and rye (R) seedlings when grown in sand and water cultures*

	AUGUST 1911		SEPTEMBER 1911		MARCH 1913		MARCH 1913		MARCH 1913		RELATIVE AVERAGE WEIGHT	
	B	R	B	R	B	R	B	R	B	R	B	R
Sand culture												
Nutrients.....	4.5	3.6	3.9	2.6	4.2	1.9	4.4	2.2	3.7	2.2	100	100
	4.3	3.6	4.2	2.5	4.0	1.9	4.5	2.2	3.8	2.1		
Nutrients plus least amount of acid.....	3.7	2.8	3.5	2.2	2.9	1.5	3.4	1.8	3.3	1.6	82	81
	3.4	2.8	4.0	2.3	3.0	1.5	3.7	1.9	3.1	1.2		
Nutrients plus largest amount of acid....	1.4	2.2	3.1	1.5	1.0	0.8	2.2	1.1	1.6	1.1	47	59
	1.3	1.8	2.7	2.0	1.1	1.0	2.8	1.3	2.3	1.5		
Water cultures												
Nutrients.....	5.3	5.6	8.1	4.5	8.4	6.2	8.2	5.6	8.8	6.7	100	100
	5.1	5.8	7.9	4.0	8.8	6.2	8.2	4.9	8.9	5.9		
	5.2	6.2	8.3	4.3								
Nutrients plus least amount of acid.....	3.3	4.7	7.1	4.2	8.3	4.3	7.4	4.1	7.7	5.6	88	84
	3.6	5.5	7.8	4.3	8.1	4.3	7.6	4.1	7.9	5.2		
	4.2	5.7	7.4	3.5								
Nutrients plus largest amount of acid....	2.1	3.7									54	60
	2.5	3.5			7.1	2.9	6.4	3.1	6.5	4.1		
		3.5			6.9	2.7	5.7	2.9	6.0	3.6		

## INFLUENCE OF THE EXTRACT OF AN ACID SOIL

That the special characteristics of an acid soil are transmitted to its extract is shown by the effect of an alkaline material, sometimes the hydroxide of sodium and again of calcium, either on the aqueous extract of soil from permanent plat 23 or on the same extract after adding nutrients. For a number of years this unlimed soil had received sulfate of ammonia, as the source of nitrogen in fertilizer chemicals. It was therefore very acid and imparted sufficient of its characteristics to the extract so that barley seedlings grew much less normally than rye seedlings, unless alkaline materials especially were added to the extract. The following numbers represent the percentage loss in weight of rye and barley seedlings caused by withholding alkaline materials from, in comparison with adding them to, the extracts of this soil:

Rye..... -2, 2, 22, 7, -10, 30, 49, 16, 36, 1; Average, 15  
Barley..... 41, 36, 44, 39, 10, 61, 57, 52, 64, 49; Average, 45

When it had been shown that rye was much less affected than barley by acid soil and its aqueous extract, whereas acidity produced artificially in sand and water cultures affected both seedlings about alike, the question arose as to whether the soil and extract contained some factor which protected the rye from the usual deleterious effect of acidity as exhibited in artificial cultures. Any treatment which would destroy such a possible agency for protecting the rye seedlings would be expected to exert a relatively greater detrimental influence on the rye than on the barley which appears not to be protected. To throw light on this point possibly, seedlings were grown in beakers and the effects noted of certain soil treatments which will now be mentioned.

Under like manurial conditions barley and rye seedlings were grown (a) in unheated soil from the unlimed sulfate of ammonia plat, (b) in the same soil after sterilization in an autoclave at about 125°C. under 15 pounds pressure for one hour, and (c) in similarly sterilized soil to which an infusion from the original soil was added subsequently. The two experiments which were

TABLE 2

*The effect on the growth of barley and rye seedlings of heating an acid soil at different temperatures*

	EXPERIMENT I RELATIVE WEIGHT OF GREEN TOPS		EXPERIMENT II RELATIVE WEIGHT OF GREEN TOPS	
	Barley	Rye	Barley	Rye
Unheated soil.....	100	100	100	100
Soil heated at 100°C.....	82	94		
Soil heated at 260°C.....	36	36	53	45
Soil heated at 360°C.....	113	94	116	90
Soil heated at 420°C.....			121	100

conducted did not furnish evidence that the hypothetical agent for protecting the rye consisted of some organism, for the soil infusion was not more beneficial to the rye than to the barley. The barley especially, however, made a very unsatisfactory growth due to the acid-soil conditions. From a similar viewpoint the soil was treated with toluol instead of being sterilized by heat, but the results do not warrant any statement of interest in connection with the idea that the rye was influenced by the association of some lower form of life which enabled it to withstand the acid conditions. Coville (2) claims that the blueberry, another acid-soil plant, has a mycorrhizal fungus associated with it "which is able to assimilate nitrogen from the surrounding organic matter, and perhaps from the atmosphere also, and to convey it into the plant without taking along with it a large amount of the poisonous soil moisture."

Soil taken from the unlimed ammonium sulfate plat in the fall of 1913 was heated at various temperatures prior to growing rye and barley seedlings in it with optimum nutrients (table 2). By the calcium-acetate method, the

amount of calcium oxide required to neutralize two million pounds of soil was 7200 pounds for the unheated soil, and for soil heated several hours at 100°, 260° and 360°C., it was 5760, 5400 and 3240 pounds, respectively: When heated for 10 hours at not less than 420°C. the amount of calcium oxide required was reduced to 2200 pounds.

Although heating the soil decreased the acidity, there was a decided increase in toxicity when the temperature of heating was around 260°C., which, however, affected the two kinds of seedlings about alike. Heating at the higher temperatures developed no toxicity, but seemed to be beneficial for barley; indicating that some substance toxic especially to barley may have had its effect reduced by the heating. In view of evidence to be presented that aluminum is such a substance, the thought arises that its availability might have been decreased by dehydration effected by the heat.

#### ADDITION OF SUBSTANCES TO ASCERTAIN IF THEY HAVE AN UNLIKE EFFECT ON THE TWO KINDS OF SEEDLINGS

Inasmuch as rye and barley are affected differently by acid soils, it was evident that some factor besides acidity must be potent. It seemed desirable, therefore, to make next the following tentative tests to ascertain whether treatment with other materials than those of an alkaline nature would have a specific effect on either kind of seedlings.

The acidity of hydrogen peroxide was nearly neutralized with sodium hydroxide; and, after the nutrients had been supplied, two amounts, 3 and 5 cc., were added to 250 cc. of soil extract of plat 23. The smaller addition of hydrogen peroxide increased the transpiration, but otherwise the barley seedlings were more abnormal with both amounts of hydrogen peroxide than when it was not added to the soil extract.

One of the poisonous organic substances which have been isolated from soil by the United States Bureau of Soils is dihydroxystearic acid. The Bureau kindly furnished a small amount of this material so that it might be ascertained whether its relative effect on barley and rye seedlings was similar to that exerted by acid soil. One hundred parts of the dihydroxystearic acid per million parts of a nutrient solution depressed the transpiration of barley 77 per cent and of rye 85 per cent; likewise, the weight of green barley tops was reduced 40 per cent and of rye tops 54 per cent. Fifty parts per million depressed the transpiration of barley and rye, 47 and 49 per cent, respectively, and the weight of green tops, 30 and 24 per cent, respectively. In this last experiment an amount of sulfuric acid equivalent to the dihydroxystearic acid depressed the transpiration of the barley 48 per cent and the weight of its green tops, 12 per cent. In another similar comparison with barley, the dihydroxystearic acid depressed the transpiration 47 per cent and the weight 28 per cent, whereas the sulfuric acid depressed the transpiration 29 per cent and the weight 13 per cent.



It appears from the foregoing data that dihydroxystearic acid does not account for the fact that barley is more injured than rye by acid-soil conditions, since it was fully as deleterious to rye as to barley. It likewise appears probable that the toxicity of dihydroxystearic acid is largely attributable to its acidity.

The relative effect of manure extract when added to an acidulated nutrient solution was tested. The manure extract was acidulated, boiled, and then neutralized before using. The development of both kinds of seedlings was depressed 56 per cent by the addition of acid to the nutrient solution and only about 42 per cent when the manure extract was also added. The barley and rye seedlings were again affected alike, however, and no evidence was obtained, therefore, that the manure extract contained any organic compound which served to account for the difference in the effect of acid soils on the two kinds of seedlings.

#### A SEARCH FOR SOME DIFFERENTIATING FACTOR IN THE SOIL EXTRACT

Half of a soil aqueous extract, with 0.0015/N acidity, was distilled from a glass retort, with the result that the distillate proved to be neutral, and the acidity of the solution remaining in the retort was double that of the original. Barley seedlings were grown with optimum nutrients dissolved in the various liquids mentioned below and the following relative averages of the transpiration and weight of the green tops secured, namely: carbon-treated distilled water, 100; soil extract, 37; distillate from the soil extract, 108; and the soil extract from which a half had been distilled, 27. The transpiration in the last instance was only a half of what it was in the original soil extract, showing that the deleterious substance simply had been concentrated in the retort and not distilled off.

The toxic factors in the extract of the soil from the unlimed sulfate of ammonia plat were not segregated by dialysis, for after a strongly toxic extract had been placed in a dialyzer for one week, the diffusate and dialyzate were shown to be equally toxic to barley seedlings. This indicated that the toxicity was of a crystalloidal nature.

Upon failure to get evidence that the toxicity was probably due mainly to organic material, it was decided to continue the investigation more especially with inorganic substances in mind.

Since nitrification would not be expected to take place normally in the unlimed plat to which sulfate of ammonia is applied as a source of nitrogen, experiments were conducted with seedlings in solution to see if rye makes relatively better use than barley of sulfate of ammonia as a source of nitrogen. If such were the case, the ability of rye to grow better than barley on the plat might be explained. It may be seen from table 3, however, that the growth of the two seedlings was depressed about alike when calcium nitrate was replaced by ammonium sulfate on the basis of equal nitrogen, that is, an

average depression of 63 per cent. This depression was doubtless due to the development of acidity where ammonium sulfate was used, rather than to this particular nitrogen combination; for, as may be seen in the table, it was necessary only to have present with the ammonium sulfate some calcium carbonate or sodium hydroxide in order to obtain practically normal growth. It would hardly be expected that under the experimental conditions there would be nitrification, and it is true that at least in some of the solutions, ammonium salts were left as such after the removal of the seedlings. The residual solutions when made up to the original volume had an average alkalinity in case calcium nitrate was used of 0.0002/N with phenolphthalein, and of 0.014/N with methyl orange as indicators; whereas when ammonium sulfate was used, instead of alkalinity there was an average acidity of 0.019/N with phenolphthalein and 0.0008/N with methyl orange. Carbon dioxide was eliminated from the solutions before using phenolphthalein as an indicator.

TABLE 3

*Relative effect of equivalent amounts of calcium nitrate and ammonium sulfate in nutrient solutions on the average of the relative transpiration and weight of green barley and rye seedlings*

	EXPERIMENT							
	I	II	III		IV		V	
	Rye	Rye	Barley	Rye	Barley	Rye	Barley	Rye
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	100	100	100	100	100	100	100	100
Ca(NO <sub>3</sub> ) <sub>2</sub> plus CaCO <sub>3</sub> .....							97	95
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	44	38	31	43	32	35	35	35
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus CaCO <sub>3</sub> .....							91	87
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> plus NaOH (varying amounts).....		54			60	55	88	78

The acidity of the solutions when sulfate of ammonia was unaccompanied by a base was sufficient to account for the depression in the growth of the seedlings.

In order to ascertain whether the ash constituents of the soil extract were in any way responsible for its different effects on rye and barley, an aqueous extract was made, and evaporated to dryness. The residue was ignited, and then dissolved in hydrochloric acid. Most of the acid was evaporated, and the remainder neutralized with sodium hydroxide. Nitrogen was supplied in the solution in such a proportion of ammonium and calcium nitrates as was necessary to control the reaction of the solution. To a portion of the soil extract, which was not evaporated, the same amounts of nitrogen and of sodium chloride were added as were present in the other solution.

Barley and rye seedlings were grown under neutral and acid conditions, not only in the above solutions, but in a regular nutrient solution. The results may be found in table 4. The results are so arranged that the development

in the neutral solutions in each of the three cases is represented by 100 so that the relative effect of the acid cultures may be seen readily by referring to the table.

Rye exhibits its usual tendency, though less than has been sometimes the case, to withstand better than barley, the toxic effect of the soil extract in its original condition. It is of importance to notice that the same tendency also exists in connection with the acid nutrient solution containing the ash constituents only of the soil extract. That is, the growth of the rye was depressed much less than that of the barley. When the acid was added to the ordinary nutrient solution, however, the growth of the rye was, in general, depressed fully as much as that of the barley; an observation which is in accord with what has usually been noticed heretofore.

TABLE 4

*The relative effect on barley and rye seedlings of an extract of an acid soil, of the ash in the same, and of an acid nutrient solution*

	EXPERIMENT I				EXPERIMENT II			
	Relative weight of green tops		Relative transpiration		Relative weight of green tops		Relative transpiration	
	Barley	Rye	Barley	Rye	Barley	Rye	Barley	Rye
Soil extract of plat 23 neutralized with NaOH.....	100	100	100	100	100	100	100	100
Soil extract of plat 23 unneutralized.....	75	74	61	66	78	92	43	67
Solution of the soil-extract ash.....	100	100	100	100	100	100	100	100
Solution of the soil-extract ash plus H <sub>2</sub> SO <sub>4</sub> .....	82	107	75	94	74	86	43	54
Nutrient solution.....	100	100	100	100	100	100	100	100
Nutrient solution plus the H <sub>2</sub> SO <sub>4</sub> .....	74	79	56	58	59	50	40	25

Evidence having been secured that some inorganic element or elements were responsible for the different effects produced by the acid-soil extract, determinations were made in the fall of 1913 of certain ingredients in an aqueous extract prepared as follows from soil of the unlimed ammonium sulfate plat. Various 2-pound portions of the soil were each mixed with 2.5 liters of distilled water, and allowed to stand about an hour, after which the liquid parts were poured off to furnish 20 liters of extract, which was then passed through a Chamberland filter. This solution contained the following in parts per million: N, 15.9; SiO<sub>2</sub>, 0.7; SO<sub>3</sub>, 65.2, and Al<sub>2</sub>O<sub>3</sub>, 27.8. The last two were present in about equivalent amounts.

The two kinds of seedlings were next grown in the presence of certain of the elements not generally considered as exerting any nutrient influence on plant growth.

The effect of even chromium was determined, because a positive qualitative test for this element was reported upon examining the aqueous extract. Potassium chromium sulfate in an acid nutrient solution failed, however, to give indications that it inhibited the growth of rye seedlings any less than that of barley seedlings.

Although manganese was absent from the soil extract, recent work at the Alabama station (3) makes it of especial interest to record that in connection with three trials of potassium permanganate as an oxidizer, the results given in table 5 had been obtained, which showed that the effect of manganese on the two seedlings was not essentially different. The basal solution contained optimum nutrients.

A trace of iron was present in the soil extract but the authors had already failed to find that this element in acid solutions affected the two seedlings differently (6).

TABLE 5

*Relative average transpiration and weights of green tops showing effect of potassium permanganate*

	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III		AVERAGE	
	Barley	Rye	Barley	Rye	Barley	Rye	Barley	Rye
No $K_2Mn_2O_8$ .....	100	100	100	100	100	100	100	100
2 parts per million $K_2Mn_2O_8$ .....	91	86	84	95			87	91
4 parts per million $K_2Mn_2O_8$ .....	87	78	89	97	90	88	89	88
8 parts per million $K_2Mn_2O_8$ .....	84	71			81	73	82	72

#### ALUMINUM AS THE CAUSE OF THE UNLIKE EFFECT OF ACID SOIL ON RYE AND BARLEY SEEDLINGS

The relative percentage depression in the average of the transpiration and weight of green rye and barley seedlings caused by adding to a nutrient solution sufficient aluminum, usually in sulfate, to produce in most cases about the same concentration of aluminum as was present in the soil extract, is shown below by different comparative tests:

Rye.....31, 30, 30, 26, 18, 18, 17, 30, 21, 34, 37, 22, 15, 19; Average, 25  
Barley.....67, 66, 62, 47, 47, 68, 62, 57, 44, 57, 46, 56, 48, 42; Average, 55

It may be seen from the preceding values that the aluminum and accompanying acidity are much less deleterious to rye than to barley; whereas it has been shown previously that the two seedlings are affected alike by acidity when unaccompanied by aluminum. In most instances the two sets of conditions, with and without aluminum, existed in the same experiment so that the differences could not be attributable to other varying conditions. Similar results were obtained also in sand cultures.

It having been shown that there exists a specific difference in the effect of aluminum on rye and barley, it seemed probable that the element either tends

to protect rye more than barley from the deleterious effect of acidity, or else it is less toxic to rye than to barley. In either case the growth of the barley would be depressed more than that of the rye.

The possibility was recognized that the association of aluminum with chromium or with silicon, two other non-nutrient ingredients of the aqueous extract, might be even more effective than the aluminum itself. A number of water cultures containing in the nutrient solution different proportions of these elements were conducted, but it was found that chromium and silicon, either with or without aluminum, exerted no unlike effect upon the two seedlings.

To decide between the two hypotheses regarding the manner in which aluminum affects rye and barley differently, it was necessary to know the comparative acidity of the nutrient solution when, to one aliquot, aluminum sulfate was added, and to another, an equivalent amount of free sulfuric acid. Under these two conditions the barley was depressed equally and the rye very unequally.

If the hydrolysis of the aluminum sulfate was insufficient to produce a degree of acidity about like that of the free sulfuric acid, then the depression in the growth of the barley must be attributable in part to the toxicity of the aluminum. Miyake (7) found that the hydrogen-ion concentration of a solution of aluminum chloride was about one-third as great as in an equivalent solution of hydrochloric acid. This led him to think that aluminum itself in some form might have been toxic, since the above-mentioned solutions with which he worked proved to be about equally injurious to rice seedlings.

In our work the nutrient solution containing aluminum sulfate was found to have about one-fourth the hydrogen-ion concentration which existed when an equivalent amount of sulfuric acid was added. This led the authors to believe that aluminum exerted its different effect because of its more pronounced toxicity to barley rather than because of an exclusive protective influence on rye.

It was recognized that extreme care was necessary in working with nutrient solutions containing such elements as phosphorus, iron and aluminum to be sure that the effect of the aluminum was not prevented by precipitation. Had this occurred in the rye cultures more than in the barley cultures, for instance, the conclusion that aluminum was less toxic to rye than to barley would have been erroneous. Owing to the difficulty of differentiating with certainty in culture solutions between a slight chemical precipitation, and turbidity naturally arising from other suspended material, certain precautions are necessary in work of this kind. Furthermore, without special precautions precipitation might take place on the roots.

When the phosphorus was reduced to only the very small amount necessary for nutrient purposes, and such proportion of ammonium nitrate and calcium nitrate used as would maintain or increase the acidity, it was felt that if a solution remained perfectly clear from standing prior to the intro-

duction of the seedlings, it was not probable that any subsequent precipitation could have taken place.

Some attempt was made to ascertain whether aluminum is toxic in neutral or alkaline solutions, using citric acid and tartaric acids or their sodium salts for the purpose of holding the aluminum in solution, even if in complex ions. Under these conditions, however, the two seedlings seemed to be affected more nearly alike, and the difficulties of knowing positively whether the aluminum had been maintained in true solution led to the abandonment of this line of work at least temporarily. It would be very desirable to know the range of circumstances in which aluminum is toxic.

TABLE 6

*The relative effect of aluminum sulfate, and its equivalent and twice this amount of sulfuric acid, on the weight of the green tops per ten barley and ten rye seedlings*

SPECIAL ADDITIONS TO THE NUTRIENT SOLUTION	WEIGHT OF GREEN TOPS			RELATIVE WEIGHT OF GREEN TOPS		
	Barley (2-rowed)	Barley (6-rowed)	Rye	Barley (2-rowed)	Barley (6-rowed)	Rye
	grams	grams	grams			
None.....	3.88 3.80	4.63 4.63	2.78 3.09	100	100	100
8 cc. 0.1/N sulfuric acid.....	2.12 2.26	3.05 2.26	1.73 1.60	58	59	59
4 cc. 0.1/N sulfuric acid.....	3.29 2.86	3.73 3.23	2.11 2.60	82	76	83
Aluminum sulfate (added sometime previously).....		3.32 2.85	2.43 2.54		67	86
Aluminum sulfate (just previously added) ...	2.59 2.51	2.91 3.04	2.09 2.72	68	65	83

An experiment typical of the many which have been conducted with aluminum salts will now be described somewhat in detail. It was conducted in the greenhouse between January 8 and 29, 1918. The results may be found in table 6. In the series where the aluminum sulfate is there mentioned as having been "added sometime previously," it was mixed with the nutrient solution a few weeks before the experiment was begun, to afford full opportunity for the aluminum to be precipitated if any of the ingredients could throw it out of solution. In the other series the aluminum sulfate was not added to the nutrient solution until just before it was supplied to the seedlings. There was no significant difference, however, in the two series.

Even if the aluminum remained in solution prior to the introduction of the seedlings, it was recognized that the nutrient solution must not become physiologically alkaline as a result of the growth of the seedlings for fear that the aluminum would be precipitated as a consequence and the purpose of the experiment be thwarted. Previous tests had taught us that the reaction of the nutrient solution may be controlled conveniently by varying the ratio of calcium nitrate to ammonium nitrate, and in the present experiment a sufficient proportion of ammonium nitrate was added so that the acidity of the solutions would at least not be reduced in the presence of the growing seedlings.

A liter of the basal nutrient solution contained 15 cc. 0.2/N  $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ , 10 cc. 0.1/N  $\text{NH}_4\text{NO}_3$ , 8 cc. 0.1/N  $\text{KCl}$ , 8 cc. 0.2/N  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 1 cc. of a solution containing 8.3 gm. of  $\text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$  per liter, and 10 cc. of a dilute  $\text{Fe}_2(\text{NO}_3)_6$  solution. In the series receiving the larger addition of sulfuric acid 8 cc. of a 0.1/N solution was also added in the liter. The alumi-

TABLE 7  
*Percentage depression caused by sulfuric acid and by aluminum sulfate*

TRANSPIRATION				WEIGHT OF GREEN TOPS			
Barley		Rye		Barley		Rye	
$\text{H}_2\text{SO}_4$	$\text{Al}_2(\text{SO}_4)_3$	$\text{H}_2\text{SO}_4$	$\text{Al}_2(\text{SO}_4)_3$	$\text{H}_2\text{SO}_4$	$\text{Al}_2(\text{SO}_4)_3$	$\text{H}_2\text{SO}_4$	$\text{Al}_2(\text{SO}_4)_3$
65	71	64	31	40	43	42	28
61	54	59	18	38	33	41	24
58	58	62	31	48	56	49	37
62	49	53	34	59	42	47	40
Average 62	58	60	29	46	44	45	32

num sulfate was made equivalent to this larger application of acid, and it may be seen that the aluminum depressed the growth of barley much more than it did that of rye, although again both seedlings were depressed alike by either concentration of acid.

Although it is obvious that the growth of barley is depressed much more than that of rye by aluminum, it is of interest to consider whether the rye is actually affected by the aluminum itself. The results given in table 7 will assist in consideration of this point. As there arranged, the percentage depression is given on both the transpiration and weight of the green tops caused in four typical experiments by the addition singly of sulfuric acid and aluminum sulfate in equivalent amounts to an optimum nutrient solution.

It may be seen that barley was affected about alike by equivalent amounts of sulfuric acid and aluminum sulfate, but that rye was depressed about twice as much by the acid as by the aluminum salt. If the latter is hydrolyzed about one-fourth as extensively as the acid, which seems to be the

case, judging from the hydrogen-ion determinations referred to previously, then not more than half the depressing effect exerted on the rye would seem to be attributable to acidity, leaving a portion of the effect to be chargeable directly to the aluminum even in case of the rye. From this point of view aluminum would seem to be some three times as toxic to barley as to rye. Only an approximate quantitative consideration, however, is warranted until more work has been done on the actual acidity of the nutrient solution as influenced by the addition of the sulfuric acid and aluminum sulfate, both before and after the growth of the barley and rye seedlings. Judging from the appearance of the roots of the seedlings, there was very little effect of the aluminum on the rye but a very marked effect on the barley (fig. 2, plate 1).

Such experiments seem to warrant the idea that aluminum itself in solution in certain acid soils may be a factor differentiating their effect on plant genera, whereas acidity from whatever source may affect the plant genera alike. Ruprecht and Morse (8) at the Massachusetts station did not discriminate as to which component of the aluminum salts was responsible for toxicity.

Investigators at the Indiana station state in connection with their work (1, p. 368) as follows:

The facts that aluminum nitrate and soil extract of the same normality with respect to aluminum are of approximately equal toxicity, and that aluminum nitrate and nitric acid of the same normality are of approximately equal toxicity, point to the acid as the toxic agent.

If in our search for a cause of the different effect of acid-soil conditions on different kinds of plants we had not rigidly excluded every toxic factor which affected alike the two kinds of plants, which were selected because of the unlike effect of acid soils upon them, it is doubtful if a probable cause would have been discovered.

One could find readily enough, many factors toxic to plants in general. An investigator is liable to fix his attention on one of these, and because of circumstantial evidence which he accumulates, conclude that he has found the cause which is responsible for the deleterious effect of acid soils.

If, however, the very genera which are affected decidedly differently by a particular set of acid-soil conditions are grown in circumstances where a specific factor is the only variant, it will be found that many of the assumed causes are only contributory to the principal reason for the effects observed in the field.

It may be found eventually that the principal disturbing factor is different under one set of acid-soil conditions than under another set, and even that the same factor may not account for the different effects in case of all genera.



## EFFECT OF MANURIAL TREATMENTS OF THE SOIL ON THE AMOUNT OF ACTIVE ALUMINUM

Inasmuch as aluminum is toxic, in connection with acid, to barley and probably other crop plants, it may be true that the improvement of certain soils by liming or phosphating is due in part to a reduction in the amount of active aluminum. To gain indications regarding this point, in the spring of 1914. samples of soil from certain permanent field plats were taken, dried, and sifted through a 1-mm. sieve. With frequent shaking for a period of three days, the soil was treated with water through which carbon dioxide was conducted in such a way as to maintain a saturated solution and atmosphere.

The following determinations were made:

PLAT NUMBER	SPECIAL FIELD TREATMENT	$\text{Fe}_2\text{O}_3, \text{Al}_2\text{O}_3,$ $\text{P}_2\text{O}_5$	$\text{Fe}_2\text{O}_3$	$\text{Al}_2\text{O}_3, \text{P}_2\text{O}_5$
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
23	Sulfate of ammonia, unlimed.....	0.063	0.007	0.056
25	Sulfate of ammonia, limed.....	0.040	0.010	0.030
27	Nitrate of soda, unlimed.....	0.044	0.010	0.034
29	Nitrate of soda, limed.....	0.036	0.010	0.026
54	Dissolved bone, unlimed.....	0.059	0.007	0.052
66	Roasted aluminum phosphate, unlimed..	0.047	0.007	0.040
68	No phosphate, unlimed.....	0.080	0.008	0.072
67	No phosphate, limed.....	0.048	0.014	0.034

The amount of aluminum and phosphorus oxides, which was determined by difference, proved to be too small for separation of the two. The first four plats received an equal liberal amount of acid phosphate annually. However, experiments at this station have shown that liming tends to increase the availability of phosphorus; consequently, if the differences are attributed to aluminum there will be at least no exaggeration. In no case had the liming been sufficient to maintain even a neutral soil reaction. Nevertheless, the results show that there was probably more active aluminum in the more acid soils, especially the very acid soil from plat 23. The four remaining plats are a part of the "phosphate experiment." The results suggest that both the phosphating and the liming may have reduced the active aluminum, even though here again the soil has never been fully neutralized. The data are recorded mainly for their suggestiveness, with a full realization of their incompleteness.

To ascertain the influence of uncombined phosphoric oxide in reducing the amount of active aluminum in soil from the unlimed sulfate of ammonia plat, 250-gm. lots of the soil were mixed intimately with water and different amounts of a solution, 25 cc. of which contained 2.5 gm. of  $\text{P}_2\text{O}_5$ , and allowed to stand for about a month, when the following was found to be soluble in 20 per cent acetic acid by digesting for 24 hours at 50 to 60°C. with occasional shaking:

	P <sub>2</sub> O <sub>5</sub>	Al <sub>2</sub> O <sub>3</sub> *
	<i>per cent</i>	<i>per cent</i>
Check soil receiving no P <sub>2</sub> O <sub>5</sub> .....	0.013	0.566
Soil receiving 1.3 gm. P <sub>2</sub> O <sub>5</sub> .....	0.015	0.524
Soil receiving 1.9 gm. P <sub>2</sub> O <sub>5</sub> .....	0.046	0.260
Soil receiving 3.0 gm. P <sub>2</sub> O <sub>5</sub> .....	0.077	0.101

\* The following percentages of certain ingredients sometimes claimed to be in a deleterious form have been found from time to time in the dry fine soil of the same general nature, which is a granitic glacial drift, namely: Al<sub>2</sub>O<sub>3</sub> 4.64, Fe<sub>2</sub>O<sub>3</sub> 3.46, Mn<sub>2</sub>O<sub>4</sub> trace, soluble in a hot, strong solution of hydrochloric acid; Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> 1.46, soluble in 0.20/N HNO<sub>3</sub> in 5 hours at 40°C.; Al<sub>2</sub>O<sub>3</sub> and Fe<sub>2</sub>O<sub>3</sub> 0.50, soluble in 0.04/N HNO<sub>3</sub>; and Al<sub>2</sub>O<sub>3</sub> 0.20, soluble in 0.013/N HNO<sub>3</sub>. Not enough iron was dissolved in the latter solvent to impart a yellow tint to the aluminum hydroxide precipitate, showing how relatively soluble the aluminum was in very dilute acid. Not more than a tenth as much calcium and magnesium oxides as aluminum and iron oxides is dissolved by the various solvents.

Indications were likewise obtained as to the effect of acid phosphate upon both the reaction and the aluminum of an acid soil, by using a sample which was taken from the unlimed sulfate of ammonia plat early in the spring of 1914. Three lots of soil of 2,000 gm. each were mixed respectively with 95, 190 and 380 gm. of acid phosphate. They were then kept moist and stirred frequently for about three weeks, when it was found by the calcium-acetate method that the calcium oxide requirement in pounds per two million pounds of soil was as follows: check soil, 4608; smallest application of phosphorus, 5472; medium, 7272; and largest, 11,880. The same soil treated in a similar manner, except that the lots stood for six months instead of three weeks, exhibited by the ammonia method<sup>2</sup> a similar increase in lime requirements with the increasing phosphate applications: namely, 7194, 9184, 11,463 and 14,783 pounds. Then the different lots of soil were placed in beakers, and nutrients were added, including a small application of mono-calcium phosphate to the soil untreated with the acid phosphate. Calcium carbonate was also added in certain beakers. Barley and rye seedlings were then grown with the average results shown in table 8, which as usual are the averages of duplicate cultures.

As usual the rye grew much better than the barley on the untreated acid soil, but not after the liming. Even the smaller amount of acid phosphate represented an application of nearly 50 tons per acre; that is, it was much larger than is made in farm practice. It is important to notice that in spite of the large amount of acidity represented by this application, the growth of the barley was markedly increased and the amount of active aluminum decreased. This reduction in active aluminum was not accompanied by an

<sup>2</sup> Submitted by the late L. P. Howard of the Rhode Island station to the 1916 Convention of the Association of Official Agricultural Chemists for publication in its journal, but not yet printed.

increased growth of rye, thus furnishing further evidence that aluminum is much less toxic to rye than to barley.

The progressive toxicity of aluminum sulfate was demonstrated by adding to 250 gm. of an unlimed fertilized soil in different beakers, 4, 8, 12, 16 and 24 cc. of a solution containing 13.665 gm. of hydrated aluminum sulfate per liter, and then growing barley. Without the aluminum, 1.90 gm. of the green tops were produced, and with the successive aluminum applications, 1.88, 1.30, 1.30, 1.15 and 0.85 gm., respectively.

Characteristic growth responses by barley and rye were observed by planting their seeds with basal nutrients in an unlimed soil from permanent plat 84, which for years had received no manurial treatment in the field. Without special treatment barley, as usual on acid soil, made a poor growth, 39 gm. of dry tops per 8-inch pot; while rye produced 60 gm., or the same as the barley after the soil was limed. The addition of 4.5 gm. of hydrated aluminum sulfate to the unlimed soil depressed the growth of barley 32 per cent and that of rye 11 per cent. An equivalent amount of sulfuric acid depressed

TABLE 8

*Results showing effect of acid phosphate on the amount of active aluminum in the soil*

SPECIAL SOIL TREATMENT	WEIGHT OF GREEN TOPS		Al <sub>2</sub> O <sub>3</sub> SOLUBLE IN DILUTED ACETIC ACID
	Barley	Rye	
	gm.	gm.	per cent
None.....	1.05	2.02	0.105
Calcium carbonate.....	2.33	1.94	
Smaller amount of acid phosphate.....	1.80	1.74	0.039
Calcium carbonate and larger amount of acid phosphate.....	1.95	1.95	0.005

the barley 40 per cent and the rye 11 per cent. Again the aluminum depressed the barley more than the rye. Probably the added sulfuric acid associated itself with sufficient aluminum in the soil so that its action was similar to that of the aluminum sulfate, for by itself the acid has been shown repeatedly to depress both kinds of plants alike.

To obtain on a larger scale the effect of thorough phosphating on plants which usually respond favorably to liming, cultures were carried on in 8-inch Wagner pots, using soil from the unlimed sulfate of ammonia plat. Four different experiments were conducted using the ordinary nutrients, including phosphorus for nutrient purposes, in each pot. When this was the only treatment both table beets and cos lettuce made practically no growth and the less sensitive barley only a small one. In the first experiment with beets, the maximum yield of roots, brought about by liming was 350 gm. per pot, obtained with about 3800 pounds of calcium oxide per acre in finely divided hydrate. In spite of treatment with probably too large an amount of acid phosphate for beets, even for the purpose at hand, at the rate of about 50

tons per acre, there was a production of 167 gm. per pot, although the acid reaction to litmus paper has been much intensified. In the second similar experiment with beets it was possible to produce only 200 gm. by optimum liming; while phosphating alone, with 14 tons of acid phosphate per acre, led to the production of 107 gm. In the third experiment 200 gm. of green lettuce leaves were produced with the optimum application of lime and 73 gm. where 14 tons of acid phosphate per acre was used. A phosphating with twice this amount, however, resulted in 272 gm., or much more than was produced by any other of a large number of treatments which were made for another purpose in the same experiment (fig. 1, plate 1). The results of the fourth experiment, with barley, are shown best by the yields of the dry, mature crop given in table 9.

The striking fact is that acid phosphate in abnormal applications, which increased the acidity of acid soils, should promote normal growth where practically no growth of very sensitive crops was possible before its application.

TABLE 9

*Results showing the effect of acid phosphate on the yield of barley*

	DRY BARLEY STRAW AND GRAIN PER POT	
	Grams	Relative weight
No special addition.....	36	100
Precipitated $\text{CaCO}_3$ , 2.75 tons per acre.....	64	178
Acid phosphate 25 tons per acre.....	92	256
Acid phosphate 25 tons, and $\text{CaCO}_3$ 2.75 tons per acre.....	105	292
Acid phosphate 50 tons per acre.....	126	350
Acid phosphate 50 tons, and $\text{CaCO}_3$ 2.75 tons per acre.....	134	372

The practical advantages of phosphating and liming may often prove to be due to the precipitation of aluminum quite as much as to supplying phosphorus as a nutrient and lime as a reducer of acidity.

#### SUMMARY

In the present study of the cause of the unlike effect of acid soils on different kinds of plants, rye was chosen as a plant which will grow well on such soils, and barley as one which is quite deleteriously affected by the same conditions.

Seedlings of these two plants were, however, affected about alike by a given amount of acidity in connection with nutrient culture both in water and in sand.

Sterilization of the soil, either by heat or toluol, did not cause any change which with certainty influenced differently the two kinds of seedlings.

Substances which affected rye and barley about alike, and therefore did not suggest the real differentiating factor, were as follows: Hydrogen peroxide, dihydroxystearic acid, manure extract, ammonium sulfate, potassium permanganate, chromium and silicon.

The aqueous extract of an acid soil affected barley and rye the same as the acid soil itself. By distillation, the toxic principle of the extract was concentrated in the residue, the distillate being nontoxic. The dialyzate and diffusate arising from dialysis of the extract both had the same effect upon the two seedlings.

The search was made among the inorganic constituents for the active factor, largely because the ash of the soil extract when brought into solution had the same effect as the acid soil.

Aluminum proved to be the element which was responsible for the different influence on the plants.

Equivalent amounts of aluminum sulfate and of sulfuric acid, when added to an optimum nutrient solution, produced about the same depression on the growth of barley. Although the sulfuric acid caused a similar depression of the rye, the aluminum salt caused very little depression, and scarcely affected the rye roots. The nutrient solution when it contained the acid was found to have about four times the concentration of hydrogen-ions as when it contained aluminum sulfate. Therefore, the toxic effect of the latter on the barley is attributable largely to the aluminum.

Treatment of an acid soil with either phosphoric oxide or acid phosphate reduced the amount of active aluminum in the soil. Unusually large additions of acid phosphate caused remarkable growths of plants so sensitive to an untreated acid soil that previously no growth was possible, and this was in spite of the fact that the acidity of the soil was very much increased by the acid phosphate. The active aluminum, however, was much decreased by the treatment.

The results indicate that the practical advantage of phosphating and liming may often prove to be due to the precipitation of active aluminum quite as much as to supplying phosphorus as a nutrient and lime as a reducer of acidity.

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# PLATE 1.

Fig. 1 (see p. 275). Cos lettuce, showing that an unusually large amount of acid phosphate was more beneficial than lime in case of an acid soil upon which lettuce could not grow. All pots received the same basal optimum nutrients.

	green lettuce gm.
1. (At left.) No special addition.....	0
2. 1500 lbs. per acre of CaO in hydrated lime.....	70
3. 2000 lbs. per acre of CaO in hydrated lime.....	145
4. 3000 lbs. per acre of CaO in hydrated lime.....	179
5. 4000 lbs. per acre of CaO in hydrated lime.....	201
6. 28 tons per acre of acid phosphate.....	277

Fig. 2 (see p. 271). Showing the like effect of acid and the unlike effect of an equivalent amount of aluminum sulfate on barley and rye. All bottles received the same basal optimum nutrients.

	Relative transpiration	Relative weight of green tops
1. (At left.) Barley with no special addition.....	100	100
2. Barley with sulfuric acid.....	31	55
3. Barley with aluminum sulfate.....	24	45
4. Rye with no special addition.....	100	100
5. Rye with sulfuric acid.....	35	54
6. Rye with aluminum sulfate.....	65	75

UNLIKE EFFECT OF ACID SOILS ON PLANTS DUE TO ALUMINUM  
BURT L. HARTWELL AND F. R. PEMBER

PLATE 1



FIG. 1

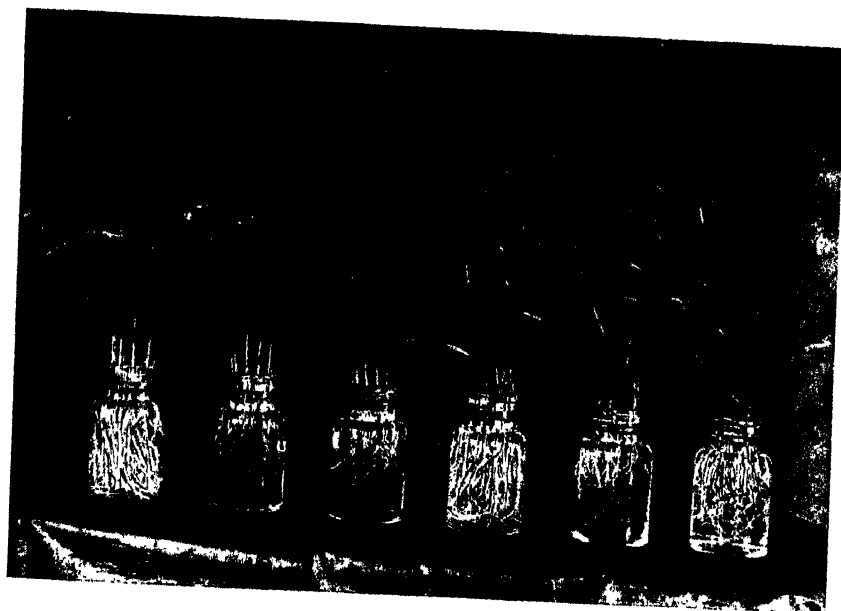


FIG. 2





# THE MOVEMENT OF PLANT-FOOD WITHIN THE SOIL<sup>1</sup>

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## INTRODUCTION

The solubility of the several plant-food elements used in fertilizers and the power of the soil to fix these elements when they are used in a soluble form as fertilizers are subjects which have received considerable attention from investigators in the past, regarding which there has been conflicting opinions, and about which there are still many things unknown. This present work is an attempt to learn, if possible, by a study of some of the fields which have been longest under experimentation, what finally becomes of the fertility elements added to the soil as fertilizers when not completely removed from the land in crops.

It has long been known that nitrogen in the form of nitrates is very often found in drainage waters, that drainage from fertile soils contains relatively smaller amounts of potassium and ammonium, and that phosphorus, when found at all, appears only in traces.

In spite of these facts, it cannot be doubted that, when used as fertilizers, the most soluble are retained to some extent in the surface soil and the most readily and firmly fixed elements gradually undergo some change and are carried deeper into the soil or are taken up by plants in a soluble form and either removed from the land or returned, again to go through the same processes.

It is a fact that should be well taken into consideration that certain substances which are generally considered insoluble are, nevertheless, washed from the soil appreciably, if not completely, by the leaching action of percolating rain-water over geological periods. It is an established fact in geological science that the comparative age of soils receiving somewhat similar amounts of rainfall may be told by the amounts of limestone which they still contain. Hilgard, after comparing the analyses of about a thousand soils from arid and humid regions, reports (21, 22) that arid soils, as a rule, contain about three times as much potassium, and twelve to fourteen times as

<sup>1</sup> This paper was submitted in partial fulfilment of the requirements for the degree of Master of Science in Agronomy in the Graduate School of the University of Illinois.

<sup>2</sup> The writer is indebted to Dr. C. G. Hopkins for helpful suggestions and criticisms in regard to the experimental work and the preparation of the manuscript.

much lime as do humid soils, and also more magnesia. Although he reports (21) no material difference in the amount of phosphorus contained in arid and humid soils, he says that there is much less organic matter in the arid soils, and since phosphorus is quite closely associated with the organic matter of soils, as shown by Stewart (42), there must be more phosphorus in mineral combination in arid than in humid soils. Although Alway found (1) but little difference in fertility elements between the Loess soils in the eastern part of Nebraska, where the rainfall amounts to about 30 inches per year, and the Loess soils in the western part of Nebraska, where the rainfall amounts to about 20 inches per year, a comparison of the content of the different fertility elements in comparable types of soils in the different glaciations of Illinois (23, 24) shows that there is a decrease from the most recently formed soil to the soil of oldest formation. With but two exceptions in the analytical data presented, giving the composition of Illinois soils, the nitrogen content of the surface seven inches decreases from the newer to the next older formed soils down through the seven glaciated areas, from 6750 pounds per acre in the late Wisconsin glaciation to 2880 pounds per acre in the lower Illinois glaciation; the phosphorus content decreases gradually, with but one exception, from 1410 to 840 pounds; and the potassium, also with but one exception, decreases gradually from 45,020 to 24,940 pounds per acre. Had the limestone not all been washed out of these surface soils, it cannot be doubted that this, too, would have shown the same gradual decrease in amount, but, after limestone is gone, the degree of limestone requirement developed depends more, perhaps, upon the nature of the remaining soil constituents than upon the amount of leaching which the soil undergoes, and yet, between the newest and the oldest soils there is a difference of 1100 pounds per acre. The data here presented (23) show that the organic matter has decreased faster than nitrogen, phosphorus or potassium, and we must assume either that the organic matter remaining is richer in these elements or that, when they have been released by the decay of organic matter, they have been fixed, partially at least, by the mineral constituents of the soil. If, however, the older soils were once as rich in these fertility elements as the newer soils now are, and there seems to be no reason for doubting it, then we must also assume either that these elements have not been completely and permanently fixed or that the mineral soil constituents with which they have united have been mechanically lost from the soil. The latter explanation does not seem sufficient, because there is but little erosion on the prairie soils of which these are types. Thus it appears that the different plant-food elements when present in soluble form are, at first, fixed by the soil and afterwards gradually leached out and washed away to some extent. That this process of loss is very slow is most vividly seen in the fact that many soils, though ages old, still contain large amounts of plant-foods, as potassium for example, and with respect to certain elements, not enough becomes soluble during a growing season to supply the needs of a large crop. One factor which operates to make the loss of plant-food slower

is the growth of the plants themselves. As reported in the several county reports for Illinois and those from Iowa and Kentucky, the nitrogen, organic carbon and usually the phosphorus are higher in the surface soil than in lower depths, especially in the soils which are level and not subject to erosion. This is not always true of potassium and calcium, there being, very often, less of these elements in the surface than in the lower depths. It has been stated that plants send their roots into the subsurface and subsoil areas where they take up plant-foods, use them to build up aerial tissues and, in nature, when they die and decay, leave these plant-foods in the surface soil. This is, no doubt, true to some extent, but the greatest activity is in the surface. By far the greatest amount of feeding takes place in the first seven inches of the soil and regardless of whether or not appreciable amounts of plant-food are brought up from lower depths, the elements which, when they become soluble in the surface soil, are taken up by the plant and rendered incapable of being leached away, will be conserved in the surface, while other materials not taken up or not rendered incapable of being washed out more easily, as potassium when the plant ripens, will be lost faster. Thus the per cent of phosphorus, for example, may increase in the surface not only because of being brought up from below, but because what is already there is retained while other materials are being decreased.

Whether or not the several factors operate to prevent the loss of materials added to soils as fertilizers, keeping them in the area of greatest activity and the best feeding range of crops, where they may become soluble or may be made available to these crops, is of prime importance to the method of fertilization. If the materials added as fertilizers are lost from the feeding zone of plants, then excesses should not be applied. On the other hand, if they remain in the feeding zone and become as insoluble as the elements placed there by nature, then why should not these elements be applied in the natural form and the effort made to bring about conditions in the soil such that they will become available to plants fast enough to insure good crops?

#### LITERATURE CITED

In order to get a comprehensive idea of the work done by other investigators which bears upon this subject, one must examine the results of experiments conducted in many ways with other objects in view than the determination of plant-food movement. Some of these will be simply drainage-water analyses, some will be irrigation studies, some will be a study of the chemical compounds found in the soil, and there are still many others. Such comment as is made upon the literature is divided somewhat into that dealing with the subject in a general way and that dealing primarily with each of the elements, phosphorus, nitrogen, potassium, calcium, magnesium and limestone.

*Literature of the subject in general*

Warrington, after a study of the drainage from soils which had received applications of soluble salts, concluded (45) that diffusion played an important part in the distribution of soluble fertilizers and other soluble salts in the soil. Later investigations do not, however, show that diffusion affects materially the movement of plant-food. Thus Müntz and Gaudechon say (32) that the soil may be considered as a discontinuous medium, that diffusion is extremely slow and that zones of very different composition may exist in the soil for very long periods. By placing soluble salts upon the surface of soil, adding water and studying the salt movement, they found that diffusion was downward and not lateral, except when the soil was drenched. Demolon and Brouet (8) agree with Müntz and Gaudechon and make the statement that there is apparently little danger of loss of nitrates in strong soil during the period of plant growth. Pettera's work (35) agrees with this, as does also the work of Malpeaux and Lefort (31). Sanborn obtained results in Utah from which he concluded (38) that there was no appreciable lateral movement of irrigation water. This may easily be true in most soils even though the horizontal movement of water in peat is very rapid, as reported by Franklin (14).

Work with lysimeters has been done by a number of investigators and in every case phosphorus is the plant-food element suffering least loss under all conditions and, when comparison has been made, less loss of all elements occurred when a crop was on the ground than when the land was fallow. Furthermore, von Feilitzen found (13) that there was less loss when the land was in grass than when in any other crop.

Robinson, after studying the results of many analyses, says (36) that phosphorus concentrates in the surface while magnesium and potassium concentrate in the subsoil.

*Literature relating to phosphorus*

There has been more work done on the study of phosphorus and more has been written on this phase of the subject than on any other. The literature of this work is voluminous and for convenience it may well be considered in accordance with the principal lines of attack.

The chemical, physical, biological, or other ways in which phosphorus is fixed, are important in that this knowledge is necessary in order to understand what must be done to increase the availability of phosphorus to growing crops. Compounds of iron, aluminum, calcium and magnesium are generally considered the ones most important in the fixation of phosphorus and it is generally believed, too, that when fixed by iron or aluminum it becomes much less available than when fixed by calcium or magnesium. Brackett and Freeman (2) found that the formation of tricalcium phosphate begins at once when ground limestone and acid phosphate are mixed. From the chemical standpoint, the phosphorus is thus made less soluble. Many trials have

been made by cultural means to determine whether lime in its several forms serves to make phosphorus more soluble or less so. Some have concluded that the tendency is for phosphorus to be made insoluble and unavailable, some believe that calcium when added to the soil, by replacing, to some extent, the iron and aluminum combined with phosphorus, makes the phosphorus more available; while still others find that the addition of lime in its various forms either has little or no effect or is beneficial in some instances and injurious in others. It is possible that not enough of the different factors are taken into account in these studies and what may appear to be the effect of calcium compound may, in reality, be due to other causes.

It has been shown that the use of fertilizer salts affects the solubility of phosphorus; those, like ammonium salts, which leave an acid in the soil tending to make phosphorus more soluble, provided, as shown by Schulov (42), the phosphate and ammonium salts are in close contact; while those which leave an alkali in the soil tend to make the phosphorus less soluble. De Jongh believes that iron and aluminum will fix phosphorus, making it insoluble and unavailable, but concludes (7) from his observations that colloids must play an important part in preventing the leaching and rapid loss from the soil of phosphates, as well as other soluble materials, at the same time holding it in such a form that it is available to plants.

Soil bacteria are also active in dissolving phosphorus and in using soluble phosphorus in their structure, thus making it unavailable to higher plants until the bacteria themselves decay.

The organic matter of soils, "humus," may fix phosphorus, as found by Dumont (9) and Karpizov (25). In this case it would again become soluble and available to plants when the organic matter itself is broken up by decay. This may be an important way in which the loss of soluble phosphorus from the surface soil is prevented. Indications that this will not always take place in all soils are furnished by the work of Petit (34).

Attempts have been made to determine the loss of phosphorus by analyzing the drainage from fertilized and unfertilized, cropped and uncropped soils. In some cases lysimeters have been used, in other cases the drainage from field plots has been analyzed. In all cases the phosphorus present in the soil, or that added, was found only in traces or not at all in the drainage, and when found at all, there was less from soil bearing a crop than from fallow soil.

In much of the investigational work, cultural methods have been used for determining the solubility of soil phosphorus. Schreiber found (40, 41) that but a small percentage of phosphorus added in a soluble form can be taken up by a crop which immediately follows, a fact which is quite commonly known. Gedroits found (15) that the order of availability of three compounds of phosphorus were: first, aluminum phosphate; second, calcium phosphate; and third, iron phosphate. Burlison found (3) that tricalcium phosphate was quite readily available to legume crops and the practice in phosphate fer-

tilizing among many farmers, especially in the middle western part of the United States, is based upon the availability of the phosphorus of tricalcium phosphate.

That soluble phosphorus is fixed in the soil must now be admitted by all, but Paturel (33) and Ullmann (44) believed that soluble phosphates remain in soluble form in the soil for long periods. This view is not generally held, and Crawley found (5) that after adding soluble phosphates to layers of soil of different depths and then leaching with water, immediately in one case and after fifteen hours in another, the amount of phosphorus held in one inch amounted to 53.35 to 99.18 per cent, while the amount held in a 6-inch layer amounted to 97.22 to 99.66 per cent. Dusserre and Bieler conducted experiments in which they found (10) that, in general, 96.6 per cent of soluble phosphorus added was retained in the first 8 cm. of soil.

In connection with this work, showing the rapid fixation of phosphorus, it is interesting to note the work of Ellett and Hill in which they find (12) that phosphorus, after being allowed to become "fixed" by calcium carbonate, magnesium carbonate, iron hydroxide or aluminum hydroxide, was yet available as a source of phosphorus to oats, wheat and corn. If this is true, it indicates that the fixation of fertilizer phosphorus in the surface soil within easy reach of plant roots is an important process and points toward the practicability of using insoluble phosphate fertilizers.

Attempts have also been made to determine the solubility of phosphorus of soils and of different phosphorus compounds in water and salt solutions, it usually being found that small amounts are soluble in pure water. Schlössing found (39) that there was more water-soluble phosphorus in a phosphorus-rich soil than in a phosphorus-poor soil, and that, in either, there was more before than after a corn crop. He found, also, that plants were able to extract more phosphorus from either a rich or a poor soil than could be extracted by water.

#### *Literature relating to nitrogen*

It may be possible that nitrogen can, in some instances, be lost from the soil in the form of ammonia, as suggested by Lemmerman and Fresenius (29) and by Grandeau (17), but soils absorb ammonia, and nitrifying bacteria readily change it into the form of nitrates, so that by far the greatest loss of nitrogen occurs as a result of the leaching out of nitrates, which are soluble. Lysimeter and field drainage analyses show that considerable amounts may be lost in this way under certain conditions, although Demolon and Brouet (8) and Malpeaux and Lefort (31) agree that the loss of nitrogen as nitrates is overestimated and that there is no great loss of nitrates, especially during the time crops are growing on the land. As mentioned in connection with analyses of soil for phosphorus, the surface soil is richer in nitrogen than are lower depths and, since plant roots can not bring nitrogen up from below, much of it, when added to soil as fertilizer or naturally by legumes, must be retained

in the surface in one or more ways. Perhaps one of the greatest factors tending to prevent its loss is the absorption of it by plants which, in their use of it, just as with phosphorus, make it insoluble; and if other plants are ready to take it up when it is liberated from old plant residues, its loss is lessened if not entirely prevented.

#### *Literature relating to potassium*

When soluble potassium is added to the soil, it is in some way held there and does not readily wash out. Dusserre and Bieler report (10) experiments in which they find that 72 per cent of the potassium added to the soil is retained in the first 8 cm. when applied at the rate of 376 pounds of the chloride per acre and leached immediately. Crawley and Duncan report (6) that 80 per cent of the potassium applied as the sulfate was retained in the first 2 inches. Others, too, have reported experiment showing the fixation of potassium, but drainage from lysimeters and from plots always shows the presence of potassium, sometimes in comparatively large amounts. It is reported that lime in its different forms, tends to make potassium soluble, but there are many experiments which indicate that the solubility of potassium may be unaffected or even decreased by lime applications. As with nitrogen, less potassium leaches into the drainage water while a crop is growing on the land, but it is known that the potassium is not so firmly fixed in the plant tissues and is partly washed out when the plants ripen, and more completely before decay is very far advanced. Rousseaux and Brioux found (37) that, while potassium was more quickly fixed in soils than was phosphorus, it was also more easily removed by leaching. The action of bacteria, as mentioned by Koch (26), is such as to render potassium more soluble, as well as phosphorus.

Taking all of these agencies into account and noting that the analyses of soils giving total amounts of potassium, as reported from various sources, do not indicate that potassium accumulates in the surface, we might expect less of the added potassium than of the added phosphorus to remain in the surface soil. Robinson concluded (36) from the results of many analyses that potassium concentrates in the subsoil. Even though it is true that potassium leaches from the soil, there are still such large amounts in most soils that if it can be made available fast enough, it is sufficient to last into the future as long as the human race need plan.

#### *Literature relating to magnesium*

Not so much attention has been given to the question of magnesium, but Collison says (4) that it appears in fairly large amounts in the drainage water from lysimeters, and Goessmann found (16) that whenever potassium chloride was used as a fertilizer, "exceptional quantities of chloride of calcium and magnesium" were present in the drainage water. The fact that more is lost



from fallow than from cropped land, as reported by Lyon and Bizzell (30), and that arid soils contain more of it than do humid soils, shows that it is subject to loss by leaching and to conservation to some extent by crops, as are other plant-foods.

### *Literature relating to calcium*

It is well known that calcium in the form of carbonate and other salts is washed from the soil. Hanamann estimates (20), that the loss amounts to from nearly 2700 to over 3500 pounds per acre from soils rich in lime, while the analyses reported by Hall and Miller (19) indicate a loss of 700 to 800 pounds per acre without ammonium fertilizers and nearly 1200 pounds per acre when ammonium salts were used.

Loss of limestone is caused not only by ammonium salts, but also by potassium and other salts which leave an acid in the soil. Crops do not take up large amounts of calcium in comparison with the amounts lost by leaching, legume crops taking up more than any other as reported by Lawes and Gilbert (28), by Hopkins (23, 24), and by others. It is to be expected that calcium and its compounds would not accumulate in the surface, and when leached from the surface would not accumulate in lower depths, because when once in solution the tendency would be for it to stay so on account of the carbon dioxide. It would then be carried on out of the subsoil into the drainage.

### EXPERIMENTAL

By determining the total amount of the various plant-food elements in samples of soil from the surface stratum and from one or two strata below the surface of plots to which fertilizers have been applied regularly for a number of years and also from comparable check plots to which no fertilizer has been applied, it would seem that one should be able to determine whether or not the fertilizer elements added have remained in the surface to increase the amount there, penetrated to the subsurface and become fixed there, or have been entirely removed from the soil by leaching and cropping.

For the purpose of making such a study, analyses were made of soil samples collected from several sections of the "Park" plots at Rothamsted, England, by Dr. J. H. Pettit, in August, 1909, and samples collected by the author through the kindness and assistance of Professors Gardner and Worthen of Pennsylvania State College, and Director Thorne of the Ohio Agricultural Experiment Station, who also furnished the author with otherwise unavailable information regarding yields of the plots sampled, from the 4-year rotation fertility plots at State College, Pennsylvania, and from the 5-year rotation plots at Strongsville, Ohio.

*The Rothamsted plots*

The collection notes for the samples taken from the "Park" grass plots will give a good idea of how and where the samples from the Rothamsted experiment fields were taken, and also something as to the treatment given each plot.

It is definitely known from records that this land has been in pasture continuously during the past 300 years. It is not known that seed has ever been sown on these plots. In 1856 experiments were started to show the effect of large amounts of various plant-food materials upon the character of the herbage. These treatments are still being continued. Topographically, the plots are extremely uniform. When sampling this uniformity was noted in the soil, as well, of the plots sampled:—Nos. 3, 4-1, 7, 9 and 12. In fact, in a letter from J. H. Pettit dated Harpenden, Eng., Aug. 26, 1909, he says—"With the exception of plot 7, I found no stones or flints at all in the first two depths, while on all plots they occurred in the third depth. In fact so regular was this that often the striking of a stone was as good an indication that the 6-inch mark had been reached as was the mark on the auger." In the early days a heavy application of chalk was made upon the north end of the plots.<sup>3</sup> Later in the eighties applications of lime were made on the west one-half of the plots. In 1903 and 1907 lime was applied to the south one-half of plots 1 to 4-2, 7 to 11-2, 13 and 16. Accordingly when plots 3, 4-1, 7, 9 and 12 were sampled in August, 1909, it was thought best to divide plot 12 north and south and plots 3, 4-1, 7 and 9 east and west and take a separate set of samples (A and B) from each half plot.

On plots 3, 4-1, 7 and 9, four rows of five borings each were made from north to south across each half of each plot, and on plot 12, two rows of ten borings each were made on each half. The samples were dried in the Rothamsted laboratory and packed for shipment.

The difference in herbage on these plots is remarkable. At the time of sampling there were no legumes on plot 9 except a single bunch of vetch on the extreme north end. Plot 7, on the other hand, was covered with legumes. Considerable quantities of sorrel were growing upon plot 9, on both the limed and unlimed ends though the growth was much ranker on the unlimed end.

Further details of the treatment given the plots sampled as reported by Lawes and Gilbert (27) are shown in table 1.

A footnote in an article published in 1880 by Lawes and Gilbert (27) reads as follows, "'Superphosphate of lime,' always composed as under, per acre; 200 pounds bone-ash, 150 pounds of sulfuric acid (sp. gr. 1.7), and water." Hall states in "An Account of the Rothamsted Experiments" (19, p. 51), that the applications of superphosphate have been 3.5 cwt. (392 pounds) per acre. It is probable that the difference of 42 pounds between this and the amount of bone-ash and sulfuric acid reported by Lawes and Gilbert is the amount of water which they also mention. Dyer estimates (11) the total amount of phosphoric acid in 50 392-pound applications of superphosphate added to Broadbalk plot No. 7 to be 3107 pounds, which would give a composition of about 7.0 per cent of phosphorus for the superphosphate. This percentage composition for the superphosphate added to the Park plots would make a yearly application of 27.44 pounds of phosphorus per acre, or, for the 54 years the plots have been under experimentation, a total application of 1482

<sup>3</sup> In sampling, this area was avoided.

TABLE 1  
*Treatment of the divisions of the plots sampled*

PLOT			REMARKS
3A	West half limed in the eighties	Lime 1903 and 1907	Unfertilized, 1856, and since
3B	West half limed in the eighties	Unlimed thereafter	
4-1A	West half limed in the eighties	Lime 1903 and 1907	Sawdust 3 years, 1856-58; superphosphate of lime, 1859 and each year since
4-1B	West half limed in the eighties	Unlimed thereafter	
7A	West half limed in the eighties	Lime 1903 and 1907	Sulfates of potassium, sodium, and magnesium and superphosphate of lime every year
7B	West half limed in the eighties	Unlimed thereafter	
9A	West half limed in the eighties	Lime 1903 and 1907	Ammonium salts in addition to the treatment given plot 7, every year
9B	West half limed in the eighties	Unlimed thereafter	
12A	Limed in the eighties	Unlimed thereafter	Unfertilized, 1856, and since
12B	Untreated		

pounds. Since sufficient analyses have not been made, or at least have not been reported, it is impossible to determine exactly the amount of phosphorus removed from each of the plots in crops. However, Hall gives (18) the average yields of the different plots for 47 years, 1856-1902 (44 years, 1859-1902, for plot 4-1).<sup>4</sup> Averages for the last ten years of this period and for 1902 alone also are given, and if we assume that the 1902 yield represents approximately the yields for the seven years 1903-1909 inclusive, the total yields of each of the plots sampled for the whole 64 years would be:

PLOT	TREATMENT	YIELD
		<i>cwt.</i>
3	Untreated.....	1109.1
12	Untreated.....	1248.1
4-1	Superphosphate alone.....	1158.2*
7	Mineral manures.....	2198.8
9	Mineral manures and ammonium salts.....	2931.2

\* See footnote 4, page 290.

<sup>4</sup> Superphosphate was not applied to plot 4-1 until 1859, sawdust having been applied in 1856, 1857 and 1858. For this reason the figure given for plot 4-1 is for only 44 years.

Report is also made of the percentage composition of the crops grown on the different plots for the 18-year period 1865-1873. These percentages are given in table 2.

TABLE 2

*Percentage composition of the mixed herbage of grass land (first crops), grown by different manures; including the composition of the pure ash-means of analyses of mixed-year samples*

PLOT NUMBER	TOTAL FOR PERIOD OF 18 YEARS, 1856-1873				
	N	P	K	Mg	Ca
3	1.66	0.160	1.115	0.194	0.867
4-1	1.58	0.282	1.096	0.199	0.838
7	1.74	0.277	2.304	0.169	0.683
9	1.55	0.333	2.139	0.149	0.428

This table shows that the crops from 4-1, 7 and 9, plots which received phosphorus fertilizer, contained an average of 1.57 times as large a percentage of phosphorus as did those from plot 3, which received no phosphorus fertilizer. If each of the crops removed from these plots contained an average of the above percentages of phosphorus, then the total number of pounds per acre removed during the period of the experiment up to and including the year 1909 were as follows:

	<i>lbs. per acre</i>
Plot 3.....	199
Plot 4-1.....	366
Plot 7.....	682
Plot 9.....	1093

The composition of the crops grown on plot 12 is not reported, nor has a division of the plots been made into the limed and the unlimed parts. However, some interesting comparisons may be made.

From the estimated additions and removals of phosphorus, even though some assumptions have been made, it is clear that more phosphorus has been added to each of the treated plots during the time they have been under experimentation than has been removed, and the difference may be as much as 389 pounds per acre on plot 9 yielding heaviest and 1116 pounds per acre on plot 4-1 which yields less than half that of plot 9. Table 3 gives the total pounds per acre (900,000 pounds) of plant-food elements as found by analyses of the samples from these plots. These show that the two halves of both plots, 3 and 12, contain about the same amounts of phosphorus in each of the depths and very much less in the surface than in the surface of the other plots. It should be noted, too, that there is less in the 3 to 6-inch stratum than in either of the other two. In the soils which have been treated, there is a nearly gradual decrease in the amount of phosphorus present from the surface down.

Since the two untreated plots contain nearly the same amounts of phosphorus in comparable depths, they may be averaged, and for like reasons, the two

TABLE 3

*Plant-food elements in the soils of the Rothamsted Park plots; average pounds per acre in 900,000 pounds of soil*

LABORATORY SOIL NUMBER	PLOT NUMBER	TOTAL ORGANIC CARBON	TOTAL NITROGEN	TOTAL PHOSPHORUS	TOTAL POTASSIUM	TOTAL MAGNESIUM	TOTAL CALCIUM	LIMESTONE PRESENT	CaCO <sub>3</sub> EQUIVALENT TO ACIDITY PRESENT
0 to 3 inch Stratum									
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
3,757	3B	46,422	3,384	522	9,918	2,394	3,987		81
3,760	3A	36,927	3,105	549	10,269	2,160	4,644	2,205	
3,763	4-1B	40,203	3,231	1,215	10,062	2,223	4,707		63
3,766	4-1A	39,150	3,195	1,260	10,467	2,358	6,435	2,709	
3,769	7B	33,687	2,835	927	11,889	2,395	2,196		99
3,772	7A	29,466	2,628	882	11,124	2,547	4,482	1,395	
3,775	9B	58,608	4,752	1,458	10,476	1,674	1,098		1,845
3,778	9A	53,019	4,086	1,395	9,738	2,313	1,881		1,035
3,781	12B	36,180	3,015	540	9,936	2,484	3,375		54
3,784	12A	38,448	3,087	567	9,819	2,367	3,177		63
3 to 6 inch Stratum									
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
3,758	3B	27,738	2,430	486	10,260	2,286	4,392		45
3,761	3A	28,071	2,376	513	10,395	2,682	3,519	963	
3,764	4-1B	27,378	2,412	828	10,287	2,286	3,789		36
3,767	4-1A	27,981	2,448	918	10,710	2,052	4,284	1,296	
3,770	7B	23,535	2,133	828	11,727	2,277	2,124		54
3,773	7A	25,317	2,178	819	10,998	2,412	3,645		36
3,776	9B	21,681	2,097	810	11,106	2,610	2,160		1,872
3,779	9A	26,892	2,394	864	10,170	2,619	1,665		1,503
3,782	12B	26,901	2,385	477	10,089	2,700	3,582		45
3,785	12A	25,407	2,313	459	10,179	2,754	3,186		36
6 to 9 inch Stratum									
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
3,759	3B	17,658	1,521	522	10,611	2,304	3,168		27
3,762	3A	19,710	1,800	540	10,773	2,079	3,042		27
3,765	4-1B	17,469	1,602	630	10,782	2,277	2,970		18
3,768	4-1A	20,709	1,782	756	10,854	2,403	3,222		18
3,771	7B	15,444	1,431	693	11,880	2,448	2,169		27
3,774	7A	19,521	1,755	657	11,007	2,574	3,159		27
3,777	9B	15,705	1,557	594	11,133	2,448	2,430		927
3,780	9A	20,925	1,863	675	10,250	2,673	2,601		990
3,783	12B	16,848	1,620	504	10,548	2,835	3,132		18
3,786	12A	16,587	1,566	531	10,647	2,385	2,394		27

halves A and B of the other plots may be averaged for the study of phosphorus. When this is done we get the figures shown in table 4.

From this table we can see how much more phosphorus the treated plots contain than the average of the untreated and, if the phosphorus added in excess of the amounts removed in crops has remained in the soil, this excess

should approximate the difference found between treated and untreated plots, provided, of course, the plots were uniform in this respect at the start. Table 5 brings these figures together.

TABLE 4  
*Pounds of phosphorus per acre (900,000 pounds)*

PLOTS AVERAGED	DEPTH OF STRATUM	PHOS-PHORUS	PLOTS AVERAGED	DEPTH OF STRATUM	PHOS-PHORUS
	<i>inches</i>	<i>lbs</i>		<i>inches</i>	<i>lbs.</i>
3A, 3B, 12A and 12B...	0 to 3	545	7A and 7B.....	0 to 3	905
	3 to 6	484		3 to 6	824
	6 to 9	525		6 to 9	675
Total .....		1,554	Total .....		2,404
4-1A and 4-1B.....	0 to 3	1,238	9A and 9B.....	0 to 3	1,427
	3 to 6	873		3 to 6	837
	6 to 9	693		6 to 9	635
Total .....		2,804	Total .....		2,899

TABLE 5

*Pounds of phosphorus per acre in treated soil over the average of that in the untreated plots 3 and 12: and calculated amount of phosphorus added to treated plots over the amount removed in crops from these plots*

PLOT NUMBER	DEPTH OF STRATUM	PHOSPHORUS BY ANALYSIS	PHOSPHORUS CALCULATED
	<i>inches</i>	<i>lbs.</i>	<i>lbs.</i>
4-1.....	0 to 3	693	
	3 to 6	389	
	6 to 9	168	
Total .....		1,250	1,116
7.....	0 to 3	360	
	3 to 6	340	
	6 to 9	150	
Total .....		850	800
9.....	0 to 3	882	
	3 to 6	353	
	6 to 9	110	
Total .....		1,345	389

The total amounts of phosphorus found in the first 9 inches of plots 4-1 and 7 over the amounts in the untreated plots, fall very close to the estimated amount added to the treated plots in excess of the amounts removed in crops.

In plot 9, there appears to be an excess of phosphorus, even in the first 3 inches, of the amount added and not removed; and for the first 9 inches there is nearly three and one-half times as much as the calculated amount in excess of that removed in crops. The cause for this cannot be determined from the data at hand. It might be possible that this plot differed from the others in this respect at the start, but that is not probable. This is the only plot receiving ammonium salts and another possible cause which suggests itself is, that by increasing the vigor of the crops they have caused the plants to send roots deeper and to absorb more phosphorus from the subsoil, leaving more of the applied phosphorus in the surface. If there has been any loss whatever from any of the plots, this loss has probably been entirely prevented from this plot. Table 3 shows that while plot 9 contains more phosphorus in the first 3 inches than either plots 4-1 or 7, it contains in the second 3 inches, as an average, less than plot 4-1 and but little more than plot 7, while in the subsoil it contains less than either of the others. This may indicate that perhaps plot 9 may contain still less phosphorus than plots 4-1 and 7 in the still lower strata, which could account for the excess in the surface 3 inches.

Comparisons of the amounts of phosphorus in the surface 3 inches show that plot 7 contains very much less than either of the other two treated plots, but the larger amounts removed in crops from plot 7 than from plot 4-1 would account for the difference between these plots. The amounts of phosphorus in the second stratum of the differently-treated plots are fairly close together and so are they for the third stratum. The fact that there is more phosphorus in the third stratum of any of the treated plots than in any stratum of the untreated plots, while, at the same time, larger crops were grown on the treated plots, shows that phosphorus has been conserved even in the third stratum of the treated plots. However, at the same rate of decrease of phosphorus content with depth as that shown in the three strata analyzed, one would not have to go much deeper to find soil of approximately the same phosphorus content as the subsoil of the untreated plots. By far the largest part of the phosphorus added to these plots, in excess of that removed in crops, has remained in the surface 3 inches, and it would seem that all of it has remained in the surface foot.

The low phosphorus content of the surface 3 inches of plot 7 suggests that the addition of alkali salts to that plot has encouraged the utilization of phosphorus by shallow-feeding plants. It is an interesting fact that, as an average, the herbage on plot 7 contains 23.8 per cent of legumes and 62 per cent of grasses, while that on plot 9 contains 0.4 per cent of legumes and 88.7 per cent of grasses. [In 1902 these percentages were 55.5 per cent of legumes and 20.3 per cent of grasses on plot 7, and 1.3 per cent of legumes and 91.2 per cent of grasses on plot 9 (18)].

Comparison shows a fairly close correlation between the content of organic carbon and of nitrogen on all plots. The surface of plot 9 is high in these two elements and plot 7 is low, showing also something of a correlation with

the phosphorus content. Plot 3B shows a somewhat abnormally large amount of both organic carbon and nitrogen in the surface, which is not easily accounted for.

In both the second and third 3-inch strata, the variations found in the surface are eliminated and there is a fair degree of uniformity over all of the plots, both treated and untreated.

The potassium content of all those plots receiving no potassium fertilizer, increases fairly gradually with depth through the three strata (table 3). The potassium added to the other plots has been in the form of potassium sulfate and was applied at the rate of 300 pounds per acre for the first 23 years, 1856-1878, and 500 pounds per acre for the last 31 years, 1879-1909, making a total of 22,400 pounds for the whole period. Commercial potassium sulfate usually contains about 40 per cent of potassium and, at this percentage, the potassium added during the 54 years has amounted to 8960 pounds per acre, where used. If the amounts of potassium in each of the strata of the different

TABLE 6

*Increase of potassium on treated soil plots due to treatment as found by soil analyses*

DEPTH OF STRATUM	PLOT			
	7B	7A	9B	9A
<i>inches</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
0 to 3	1,903	1,138	490	-248
3 to 6	1,496	767	875	-61
6 to 9	1,235	362	488	-394
Total.....	4,634	2,267	1,853	-703

untreated plots 3B, 3A, 12B and 12A are averaged, the following pounds per acre are found:

0 to 3-inch.....	9,986
3 to 6-inch.....	10,231
6 to 9-inch.....	10,645

If, now, we subtract from the potassium content of each of the different strata of the treated plots the potassium content of the corresponding stratum of the untreated plots, we will get an estimated amount of potassium increase due to treatment. These amounts are given in table 6.

If that part of the added potassium which has not been removed in crops has remained in the surface 9 inches of soil, then the total amounts given in the above table should agree with the amounts added in fertilizer in excess of that removed in crops. Unfortunately for this comparison, we do not have the yields of each half of the plots separate, but for the whole of plot 7, the calculated amount added in excess of that removed has been 3286 pounds per acre, while that for plot 9 has been 1938 pounds. The amount for plot 7, 3286



pounds, approximates fairly closely the average gain in potassium of the two halves of plot 7, 3450 pounds, but it will be noticed that the actual increase of potassium in plot 7 B, without lime, is 1348 pounds above the calculated amount, while the actual increase for plot 7A, with lime, is 1019 pounds less than the calculated amount. The agreement between the actual and the calculated increase for plot 9B, receiving ammonium salts without lime, is close, but the actual increase is slightly lower, and plot 9A receiving both ammonium salts and lime, contains 803 pounds of potassium less than do the check plots while, calculating from the applications and the crops removed, there should have been an increase of approximately 1938 pounds.

In plot 9B, receiving ammonium salts and in plot 9A receiving ammonium salts and lime, there is an increase in potassium content in going from the first to the second stratum, much the same as in the untreated plots, while in plot 7B, and in plot 7A to a less extent, there is more potassium in the first than in the second stratum. Those plots which have received potassium fertilizers and show an increase in potassium content, show an increase in the second and third strata, while the plot showing a decrease, shows a greater decrease in the third stratum than in either the first or the second.

The conclusion that must be drawn is that though potassium is fixed in the surface, its loss is easily brought about by methods of treatment. Table 3 shows, however, that if there are means of making the potassium available, there is room for many times the depression suffered by any plot before it approaches exhaustion.

The magnesium content of the different plots bears no apparent relation to the applications made. If the magnesium sulfate added contains 10 per cent of magnesium, the total application of magnesium has amounted to only 540 pounds for the 54 years. The crops removed from the plots receiving magnesium contain a lower percentage of this element than do the check plots, though the absolute amounts removed from the treated plots have been about twice that removed from the check plots. Perhaps the most noteworthy point is that where ammonium salts have been used, the magnesium in the surface 3 inches has been decreased, even in spite of the application of magnesium fertilizers. This is particularly true of plot 9B. Other than this, there seems to be but little that can be said, since the amount of magnesium added is small and the variation in content of magnesium is greater within those plots receiving none of it than between those receiving it and those receiving none, excepting plot 9B.

It has already been quite definitely shown that the tendency for calcium is toward a gradual movement downward and out of the soil. Table 3 shows that the calcium content corresponds very well to the content of carbonate or the acidity developed. Plot 4-1B seems to be an exception to this, but even in this plot the amount of calcium is not high and the acidity developed is not great. Although, as stated in the collection notes, lime was applied to the west half of the plots in the early eighties, the calcium and acidity

content of plot 12A and 12B indicate that the east and the west halves have again become uniform in this respect. The effect of the lime applied in 1902 and 1907 is still visible on all plots. There is not more calcium in the soil of plot 3A than in that of plot 4-1B which has not received lime, but plot 3A contains carbonates in each of the first two strata, while plot 4-1B has a greater lime requirement in the surface than in either of the other two strata.

Plots 3A, 4-1A and 7A show that so long as applied limestone remains in the soil at all, to the depths sampled and very probably to much greater depths, by far the greatest amount of it is in the surface. That it does penetrate to the second stratum is shown, however, by plots 3A and 4-1A. The effect of ammonium salts in causing a loss of both calcium and carbonates is well illustrated on plots 9B and 9A. Either of these plots has a much greater lime requirement in all three strata than has any of the other plots and the calcium content in the surface of either is lower than that of any other plot, even though lime has been applied to plot 9A. This lime has, however, made the lime requirement of plot 9A considerably less than that of plot 9B. It appears from plot 7B that the mineral salts used have caused a loss of calcium in all three strata without having very materially increased their acidity.

In so far as the results of tests made on these Park plots show, the movement of the different elements, even downward, is very slow. The practice which is followed in adding fertilizers indicates that there is no appreciable lateral movement. Adjoining plots are not separated by division strips, but when fertilizers are applied, a canvas sheet is put up along the division line to prevent the blowing of material upon plots where it should not be and, as shown by the plot yields, one plot receives no benefit from the fertilizers added to any of the adjoining plots.

### *The Pennsylvania plots*

The first of the experimental crops were removed from these plots in 1882, so that 1915 completed the 34 years for which we have records of the yields and treatment given to each plot. A diagram of the plots and the treatment which each has received are given in the annual report of the Pennsylvania State College for 1911-1912, p. 84-86. The plots chosen for study were plots 1 and 14, both check plots receiving no treatment, plot 3 receiving only phosphorus, and plots 9 and 17 receiving full treatment of nitrogen, phosphorus and potassium. Each of these plots on each of the four series (I, II, III, IV) were sampled in three depths, 0-6 $\frac{1}{2}$  inches, 6 $\frac{1}{2}$ -13 $\frac{1}{2}$  inches and 13 $\frac{1}{2}$ -20 inches, about eighteen borings to each plot, each depth being kept separate and all analyses made on each sample separately.

The samples taken from the treated plots appeared to be very much like the check plots with which they are compared. Because of some non-uniformity in plot 14, series 4, part of the plot was left unsampled. By doing so, a better check sample with which to compare samples from plots 9 and 17 was obtained.

The yields of these plots were obtained from the annual reports of the Pennsylvania State College for the years 1901-1902, 1907-1908, 1911-1912 and from yields furnished by Professor Worthen. Table 7 gives the total number of applications of fertilizers made, the rate and the total amounts of the three elements, nitrogen, phosphorus and potassium. Table 8 gives the total

TABLE 7

*Fertility treatment given the Pennsylvania plots, Series I, II, III, IV*

PLOT NUMBER	NUMBER OF APPLICATIONS	ELEMENTS APPLIED	RATE, POUNDS PER ACRE	TOTAL POUNDS PER ACRE
1				
3	17	P	20.96	356
9	17	N	24.00	408
9	17	P	20.96	356
9	17	K	83.00	1,411
14				
17	17	N	24.00	408
17	17	P	20.96	356
17	17	K	83.00	1,411

TABLE 8

*Pounds per acre of phosphorus removed from each plot in crops*

SERIES NUMBER	PLOT 1	PLOT 3	PLOT 9	PLOT 14	PLOT 17
	lbs.	lbs.	lbs.	lbs.	lbs.
I	131.6	203.8	259.0	176.6	276.6
II	159.4	226.7	312.6	226.1	260.5
III	147.2	228.5	284.9	166.6	250.1
IV	198.3	234.5	302.5	166.3	257.5

TABLE 9

*Net gain (+) or loss (-) of phosphorus per plot, 1882-1915, as a result of fertilizing and cropping*

SERIES NUMBER	PLOT 1	PLOT 3	PLOT 9	PLOT 14	PLOT 17
	lbs.	lbs.	lbs.	lbs.	lbs.
I	-132	+152	+97	-177	+ 79
II	-159	+129	+43	-226	+ 95
III	-147	+127	+71	-167	+106
IV	-198	+121	+53	-166	+ 98

amounts of these three elements removed from each of the plots in crops, taking as the average amounts of the three elements in the different crops, the amounts given by C. G. Hopkins, in "Soil Fertility and Permanent Agriculture," p. 154. Table 9 gives the net gain or loss of phosphorus as a result of fertilizing and cropping.

If, at the beginning of the field experiments, each of these Pennsylvania plots had contained the same amount of phosphorus in each of three strata sampled, and if the crops removed from all plots had contained the same percentage of phosphorus, then it would be an easy matter to determine the proportion of added phosphorus remaining in each of the layers and to trace its movement downward. Thus a comparison of plots 1 and 3 in series III shows that plot 1 has had 147 pounds of phosphorus removed from it in crops with no additions made, while plot 3 has had 127 pounds more phosphorus added in fertilizers than removed in crops, thus making a difference of 274 pounds which plot 3 should contain more than plot 1. Analyses of these two plots show that plot 3 contains, in the first stratum ( $6\frac{3}{4}$  inches), 240 pounds more phosphorus than plot 1; in the second stratum 20 pounds, and in the third stratum 20 pounds, a total of 380 pounds which very closely approximates the difference of 274 pounds found by the above estimation. In this instance it would be indicated that on plot 3 about 86 per cent of the added phosphorus plus that available in the soil, as indicated by the amount removed from check plot 1, has been retained in the first  $6\frac{3}{4}$  inches or the plow depth, while the next two strata of like extent contain only about 7 per cent each.

It is not safe to rely too much on this assumption, however, and, unfortunately, we do not have the phosphorus content of these three strata at the beginning of the field experiments nor the composition of the crops removed from the different plots. In general, however, the phosphorus content of the different plots must have been similar, though there may have been some variation, and for the present we can not do better than to use an average composition for all crops.

While this comparison can not be accurately made, table 11 is made up in such a way as to show the difference between check and treated plots as calculated from the amounts removed in crops and the amounts added in fertilizers, also the amounts found by analyses in treated plots more than in check plots (+) or less than in check plots (—) in each of the sampled strata. The total amounts of phosphorus found in the different strata are given in table 10.

Although there is but one comparison in which the two figures agree so well as in the above-mentioned case, we are able to obtain some quite definite and valuable information which points qualitatively, if not quantitatively, to the same conclusion as that reached in considering plots 1 and 3, series III.

Plots 1 and 3 being located nearer each other than any other check and treated plots, these were first considered on all the series, and for the same reason plots 14 and 17 were next taken. Plot 9 was compared with the average of check plots 1 and 14.

Comparison shows that in only one case does a check plot contain more phosphorus in the surface stratum than does a treated plot with which it is compared, on series I, plots 9 and 14. In all other cases the treated plot contains from 100 to 400 pounds, or from 11.1 to 54.3 per cent, more phos-

phorus than the untreated plot compared with it. In the subsurface, the phosphorus content of the check plot is, in four of the twelve comparisons, higher than the treated plots with which they are compared, while the dif-

TABLE 10

*Pounds of phosphorus pe acre (2,000,000 pounds) in the soils from Pennsylvania, by analysis*

PLOT NUMBER	0 TO 6½ INCH STRATUM	6½ TO 13½ INCH STRATUM	13½ TO 20 INCH STRATUM	TOTAL
Series I				
	lbs.	lbs.	lbs.	lbs.
1	900	660	580	2,140
3	1,140	760	600	2,500
9	960	700	620	2,280
14	1,080	880	680	2,640
17	1,400	1,100	800	3,300
Series II				
1	900	660	660	2,220
3	1,000	720	640	2,360
9	1,140	820	700	2,660
14	920	880	780	2,580
17	1,320	800	760	2,880
Series III				
1	860	680	660	2,200
3	1,100	700	680	2,480
9	1,140	820	600	2,460
14	780	620	620	2,020
17	1,040	720	620	2,380
Series IV				
1	900	760	660	2,320
3	1,100	720	640	2,460
9	1,080	680	580	2,340
14	700	620	560	1,880
17	1,000	740	640	2,380
Average for all series				
1	890	690	640	2,220
3	1,085	725	640	2,450
9	1,080	755	625	2,435
14	870	750	660	2,280
17	1,090	840	705	2,735

ference found in the other eight comparisons vary only from 20 to 170 pounds, or from 2.9 to 26.1 per cent. In the subsoil, seven of the twelve comparisons show that the check plots contain more phosphorus than do the treated plots

with which they are compared, while the other five comparisons show the narrow variation of from 0 to 120 pounds, or 0 to 17.6 per cent.

Averaging the percentage differences between treated and check plots for all four series, comparison of plots 1 and 3 shows a difference in phosphorus content in favor of the treated plots amounting to 22.0 per cent in the first

TABLE 11

*Phosphorus per acre in treated plots more than in untreated plots by analysis and calculated increase per acre of phosphorus in treated plots over untreated plots*

PLOTS COMPARED		INCREASE OF 3 OVER 1		INCREASE OF 17 OVER 14		INCREASE OF 9 OVER AVERAGE OF 1 AND 4		AVERAGE INCREASE FOR THE THREE COMPARISONS	
Series	Stratum	By analysis	Calcu- lated	By analysis	Calcu- lated	By analysis	Calcu- lated	By analysis	Calcu- lated
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
I	1	240		320		-30		177	
	2	100		120		-70		50	
	3	20		120		-10		43	
		—		—		—		—	
	Total.....	360	284	560	256	-110	251	273	264
II	1	100		400		230		243	
	2	60		-80		50		10	
	3	-20		-20		-20		-20	
		—		—		—		—	
	Total.....	140	288	300	321	260	191	233	267
III	1	240		260		320		273	
	2	20		100		170		97	
	3	20		0		-40		-7	
		—		—		—		—	
	Total.....	280	274	360	273	450	229	363	259
IV	1	200		300		280		260	
	2	-40		120		-10		23	
	3	-20		80		-30		10	
		—		—		—		—	
	Total.....	140	319	500	264	240	235	293	273
Average	1	195		320		200		238	
	2	35		65		35		45	
	3	0		45		-25		7	
		—		—		—		—	
	Total.....	230	291	430	279	210	227	290	260

6 $\frac{2}{3}$  inches, of 5.7 per cent in the second 6 $\frac{2}{3}$  inches, and of only 0.1 per cent in the third 6 $\frac{2}{3}$  inches. Corresponding averages for comparisons of plots 14 and 17 are 35.4, 12.9 and 7.3 per cent; and for the 1 and 14 average and 9, 22.7, 4.9 and -3.8 per cent. Thus it is seen that the addition of phosphorus fertilizer has, after 34 years of fertilizing and cropping, left the surface of the treated plots 26.7 per cent richer in phosphorus, as an average of all above

comparisons, than the untreated plots with which they are compared, the average difference in the subsurface being but 7.8 per cent while the average of the subsoils of the treated and the untreated plots show practically the same phosphorus content, being only 1.2 per cent in favor of the treated plots.

From these data it appears that a large part, perhaps three-fourths, of the added phosphorus has remained in the plowed soil or has been removed in crops, that nearly, if not quite, all of the remainder has not been carried lower than  $13\frac{1}{2}$  inches. Results would be still more in favor of this conclusion if, as shown by analyses of crops from the Park plots, the crops from treated land contains more phosphorus than those from the check plots, and if this had been taken into account in the above calculations.

### *The Ohio plots*

Samples taken from the Strongsville plots were made up of composites from about ten borings and represent the same strata as those from Pennsylvania. Samples were taken from corresponding plots on series A, B, C and D, the plots being 1, 4, 10, 13, 16 and 19, all check plots receiving no treatment; plot 2 receiving phosphorus at the rate of 20 pounds per acre in 5 years; plot 11 receiving phosphorus at the rate of 20 pounds, nitrogen at the rate of 76 pounds and potassium at the rate of 108 pounds in 5 years; and plot 17 receiving these three elements at the rate of 30, 38 and 108 pounds, respectively, during the same period. Table 12 gives the total amount of these elements added from the time the experiment began to the time the samples were collected. Table 13 gives the total amount of phosphorus in each stratum sampled, as found by analyses, and table 14 shows the estimated removal of phosphorus in crops if we consider all crops to have the amount used by Hopkins (23, 24) as the average composition.

It will be seen by comparing tables 12 and 14 that in no case has as much phosphorus been added in fertilizers as has, according to this estimate, been removed in crops. If the crops from treated plots contained a larger percentage of phosphorus, the difference is still greater.

Even though more phosphorus has been removed than returned to the treated plots, that added should conserve the phosphorus originally in the soil so as finally to make the treated plots higher in this element than the check plots, unless the amount removed in crops from the treated plots is enough to equal that added in fertilizers plus that removed from the check plots. Table 15 shows the gain or loss in phosphorus in pounds per acre for treated plots over the weighted average of the check plots on each side of it, as found by analysis of the soil samples. It gives, also, the calculated amount of phosphorus the treated plots should contain more than the weighted averages of check plots based on the above calculation.

This table shows that the calculated increase of the various treated plots over the untreated ones is very small, amounting in many instances to less than the limit of analytical error, which is 40 pounds. If the treated plots produced crops containing a higher percentage of phosphorus than the average here used, and the check plots produced crops with a lower percentage composition than the average used, it might easily be that an actual loss of phosphorus would be shown for the treated plots in comparing them with the check plots, as is shown by the analytical results in several instances. With such small differences and the variations found in untreated plots, which is greater in many cases than the variation between comparable treated and

TABLE 12

*Amounts per acre of fertilizer elements added to the Strongsville plots*

	PLOT	P	K	N
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
Series A.....	2	80		
	11	80	448.56	304
	17	110	448.56	117½
Series B.....	2	80		
	11	80	432.00	304
	17	115	432.00	164½
Series C.....	2	80		
	11	80	390.46	278½
	17	110	390.46	139½
Series D.....	2	80		
	11	80	456.92	299½
	17	120	456.92	201½

untreated plots, nothing better can be done than to compare the amounts in the different strata as shown in table 13. In general, much more phosphorus is found in the surface than in either the second or the third stratum. In some cases the next largest amount is in the second, and in other cases, in the third stratum. The sample from the second stratum of plot 17, series B, and from the third stratum of plot 10, series C, are abnormally high, possibly because of the inclusion in these samples of phosphatic material that is there as a contamination from some unknown source. Otherwise, there is less fluctuation in the content of phosphorus in the two lower strata of the different plots than there is in the surface stratum.



TABLE 13

*Pounds per acre (2,000,000 pounds) of phosphorus in the soil samples from the Ohio plots*

PLOT NUMBER	0 TO 6 $\frac{1}{2}$ INCH STRATUM	6 $\frac{1}{2}$ TO 13 $\frac{1}{2}$ INCH STRATUM	13 $\frac{1}{2}$ TO 20 INCH STRATUM	TOTAL
Series A				
	lbs.	lbs.	lbs.	lbs.
1	820	340	480	1,630
2	920	420	440	1,780
4	740	400	420	1,560
10	1,020	280	280	1,580
11	1,060	440	360	1,860
13	740	640	340	1,720
16	740	380	360	1,480
17	1,000	440	360	1,800
19	1,020	480	380	1,880
Series B				
1	920	400	520	1,840
2	820	440	480	1,740
4	720	340	600	1,660
10	1,000	520	480	2,000
11	1,100	400	320	1,820
13	1,000	500	360	1,860
16	740	440	340	1,520
17	900	880	320	2,100
19	1,020	520	420	1,960
Series C				
1	860	340	360	1,560
2	820	400	340	1,560
4	860	440	340	1,640
10	920	460	1,340	2,720
11	1,040	580	400	2,020
13	1,160	640	420	2,220
16	820	320	320	1,460
17	920	400	380	1,700
19	900	700	340	1,940
Series D				
1	1,160	740	420	2,320
2	1,000	400	380	1,780
4	1,160	620	480	2,260
10	940	360	280	1,580
11	760	320	300	1,380
13	900	320	460	1,680
16	960	360	380	1,700
17	900	380	380	1,660
19	960	480	340	1,780

TABLE 14

*Calculated amount of phosphorus per acre removed in crops from the Ohio plots*

PLOT	SERIES			
	A	B	C	D
	lbs.	lbs.	lbs.	lbs.
1	67.7	64.5	62.2	82.6
2	117.0	86.3	94.9	128.5
4	61.6	42.7	57.0	84.6
10	77.9	83.1	70.9	85.3
11	140.9	119.3	101.9	128.7
13	75.9	71.7	69.2	94.4
16	85.5	72.0	76.4	94.7
17	139.7	116.5	114.6	139.5
19	91.2	81.7	56.7	90.6

TABLE 15

*Pounds per acre gain (+) or loss (-) in phosphorus of treated over weighted average of untreated plots*

PLOT NUMBER	UM	SERIES								AVERAGE	
		A		B		C		D			
		By analysis	Calcu- lated	By analysis	Calcu- lated	By analysis	Calcu- lated	By analysis	Calcu- lated.	By analysis	Calcu- lated
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
2	1	+133		- 33		-40		-160		- 25	
	2	+ 60		+ 60		+27		-300		- 38	
	3	- 20		- 67		-13		- 60		- 40	
	Total..	+173	+28.7	- 40	+50.9	-26	+45.6	-520	+ 34.8	-103	+40.0
11	1	+133		+100		+40		-167		+ 27	
	2	+ 40		-113		+60		- 27		+ 10	
	3	+ 60		-120				- 40			
	Total..	+233	+16.3	-133	+40.0		+48.1	-234	+ 39.6		+36.0
17	1	+167		+ 67		+73		- 60		+ 62	
	2	+ 27		+413		-47		- 20		+ 93	
	3	- 7		- 47		+53		+ 13		+ 3	
	Total..	+187	+57.7	+433	+73.7	+79	+65.2	- 67	+104.0	+158	+75.1
Average	1	+141		+ 45		+24		-129		+ 21	
	2	+ 42		+120		+13		-116		+ 15	
	3	+ 11		+ 78				- 29			
	Total..	+194	+34.2	+243	+54.9		+53.0	-274	+ 55.9		+50.4

## CONCLUSIONS

The conclusion must be drawn from the work presented here that when phosphorus is used as a fertilizer, it remains almost where it is placed in the soil until removed in crops or removed by some such process as erosion by water or wind action.

The addition of alkali salts (sulfates of potash, soda and magnesia) seems to encourage the utilization of phosphorus from the surface stratum, especially by legume plants, which probably also secure nitrogen chiefly from the soil air in the surface stratum.

There may be some loss of nitrogen through drainage, but when other fertility conditions are right and crops are kept on the ground all through the growing season, this loss is very small and there is a tendency for nitrogen, added in the form of ammonia, to accumulate in the surface soil, probably in plant roots and residues.

Potassium, though easily and quickly fixed in soil, is more subject to movement within the soil as a result of fertilizing with other salts, and in this way may be leached beyond the reach of plant roots.

Carbonates are rather easily washed from the soil even when no other treatment is given, but much more readily washed out when ammonium salts are used.

The loss of magnesium is brought about by the use of ammonium salts as fertilizers.

Calcium decreases with the loss of carbonates. Also, when alkali fertilizer salts are applied, it decreases more rapidly than acidity develops. Ammonium salts cause as marked a loss of calcium as of carbonates, and its loss occurs relatively as rapidly as the acidity develops.

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# THE OCCURRENCE OF ACTINOMYCETES IN THE SOIL

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## INTRODUCTORY

Actinomycetes include both parasitic and saprophytic forms and can be isolated from air, water, sewage, salt lakes, milk and certain wounds, but chiefly from the soil. They form a large and important group of soil micro-organisms; this is manifested by the total numbers of these organisms found in different soils, numerous species and manifold activities. Attention has been repeatedly called to the fact that in soil containing an abundance of undecomposed organic matter, such as the stubble and roots of plants and animal manures, the actinomycetes are present in large numbers greatly exceeding those in the corresponding soil poor in undecomposed organic substances. As is pointed out elsewhere (24), these organisms have such a variety of activities that they cannot be spoken of as a group, as one cannot speak of the bacterial activities as of one group, but rather of a number of species, whose functions are variable.

## HISTORICAL

Globig (6) described in 1888 a thermophilic Actinomyces, isolated from garden soil upon potato as a medium, having an optimum temperature of 58°C. Rossi-Dóri (20) isolated an Actinomyces from the soil that he called *Streptothrix alba*, a term commonly applied to many soil actinomycetes.

Rullman (21) studied one form in 1895 which he called *Actinomyces odorifer*, due to the production of the peculiar odor so characteristic of this whole group.

Beijerinck (1) found actinomycetes one meter deep in garden soil, two meters deep in sandy soil and in the mud of the Meuse under the river bed. He stated that these organisms are omnivorous and can live and reproduce under circumstances favorable and even unfavorable for nutrition; he also pointed out their ability to decompose organic substances, since they produce quinone, which is a carrier of oxygen and further oxidation.

Nadson (18) isolated in 1900 three Actinomyces from the curative mud of a salt lake in Russia, and studied them in detail, but unfortunately his paper seems to have been overlooked by subsequent investigators. He stated that they decomposed proteins readily with the production of ammonia and hy-

drogen sulfide; they are also characterized by the precipitation of  $\text{CaCO}_3$  in large quantities.

Hiltner and Störmer (13) found in 1903 that in the fall there is an increase in the numbers of actinomycetes relative to the other groups of microorganisms due to the increase of the content of undecomposed organic matter in the soil. They form in the spring 20 per cent, in the fall 30 per cent, and dropping in the winter to 13 per cent of the total soil microbial flora; the addition of stable manure, due to its straw content, results in an increase in the numbers of actinomycetes.

Petri (19) isolated an *Actinomyces* from the roots of strawberries and expressed the opinion that the actinomycetes are energetic destroyers of organic matter; he has shown that they can be inoculated into plants but that they act as saprophytes, since after six months the plants were still in a healthy condition.

Gilbert (5) in 1904 isolated an *Actinomyces* from the soil, with potato as a medium, which grew at an optimum temperature of 50–55°C. Hiltner (12) found the actinomycetes to occur in large quantities in stable manure and ascribed to them the importance of fixing in their bodies the easily assimilable nitrogen compounds. Heinze (10, 11) speaks of the actinomycetes as playing an important part in soil fermentation. Macé (16) isolated an *Actinomyces* from the soil which was found to be one of the most active agents in the transformation of albuminous matter. Long before that (15) Macé reported about an *Actinomyces* found in abundance in water which is able to deposit calcareous secretions around its filaments.

Fisher (7) found in sandy, sandy loam and loamy soils not more than 15 per cent of the microorganisms to be actinomycetes.

Fousek (8) found that loam soils contain a higher number of actinomycetes than other soils, sandy soils coming last. He confirmed the observation of Hiltner and Störmer (13) that there is a proportionate increase of actinomycetes in fall (27 to 35 per cent) than in the spring (18 to 23 per cent), but there is no reduction in the winter. Cultivation of the soil effects a decrease in the numbers of actinomycetes; the numbers are higher in the fall because new quantities of undecomposed organic substances are added to the soil. Actinomycetes were found in forest soils and on the roots of different plants, particularly grass and legume roots, the upper cell layers of which died down. He therefore concluded that the actinomycetes are important in the decomposition of plant residues in the soil. When cultures of actinomycetes were added to the soil, a better growth of important agricultural plants was obtained because the organic substances of the soil are decomposed energetically by these organisms, thus making available larger quantities of nutrients, particularly nitrogenous compounds, for the plants. He suggested that the actinomycetes stimulate nodule formation on the roots of leguminous plants, attacking these roots, resulting in a more ready access for the legume bacteria. Hagem (9) isolated four actinomycetes and Münter (17) isolated seven species

from the soil. Conn (2) stated in 1913 that the actinomycetes may make up as many as 40 per cent of the soil bacteria. While Hiltner and Störmer (13) found as many as 2.5 millions of actinomycetes per gram of soil and Conn (2) 12 to 14 millions, Krainsky (14) obtained only 20,800 per gram, but these formed 30 per cent of the total number of microorganisms developing on the special medium used. Krainsky concluded that the actinomycetes play an important part in the decomposition and humification of plant remains in the soil. The senior writer (22) found that the actinomycetes are present in large quantities in all cultivated and uncultivated soils. Their numbers decrease with depth of soil but increase in proportion to the other soil microorganisms.

Conn (3) observed a greater number of actinomycetes in old sod soil (39.4 per cent of the microbial soil flora) than in cultivated soil (21.3 per cent). He suggested the idea that these large numbers in sod soil are due to the fact that the actinomycetes are concerned in the decomposition of grass roots. Conn (3) brought further evidence that an addition of grass roots to soil resulted in an increase in numbers of actinomycetes. This only confirms the observations previously made on the same point by Hiltner and Störmer (13), and Fousek (8).

The writers (25) found an average of 743,000 actinomycetes at a depth of 1 inch in New Jersey soils and 933,000 at a depth of 4 inches, which formed 9.2 and 15.0 per cent, respectively, of the total microbial soil flora with the culture media used. The numbers of actinomycetes regularly decrease with depth, but increase in proportion to the other microorganisms, so that at a depth of 30 inches they formed an average of 240,000 per gram, or 65.6 per cent of the total microbial flora developing on the given media. Similar results were obtained with an Oregon soil, while several California soils studied gave 380,000 to 1,890,000 of actinomycetes per gram, or 19.2 to 45.0 per cent of the total microbial population found with the given medium.

Conn (4) criticizes the work of the writers and thereby allows several unpardonable mistakes to creep in. The medium most extensively used in our work was Czapek's solution agar, which is well known to mycologists, and the description of its composition in the paper was thought to be superfluous, particularly since it has been recently given by a number of investigators in their different publications.<sup>1</sup> This very formula was given in a personal letter to Dr. Conn. The composition of this medium is given on page 313. But Dr. Conn ascribed to us the addition of 0.2 per cent  $\text{KNO}_3$  to the Czapek's

<sup>1</sup>Dox, A. W. 1910 The intracellular enzymes of *Penicillium* and *Aspergillus*, with special reference to those of *Penicillium Camemberti*. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 120, p. 37.

Thom, C. 1915 The *Penicillium Luteum*-*Purpureogenum* group. In *Mycologia*, v. 7, p. 139.

Scales, F. M. 1914 The enzymes of *Aspergillus terricola*. In *Jour. Biol. Chem.*, v. 19, p. 461.



solution and the substitution of dibasic for monobasic potassium phosphate; and also, since Thom referred to a certain modification of Czapek's solution made by Dox as Dox's solution, Dr. Conn labels our Czapek's solution agar as Dox's agar. This is entirely incorrect, since the medium used by us has been referred to also by many others as Czapek's solution agar.

Dr. Conn further criticizes the fact that we laid a great deal of emphasis upon the liquefaction and pigment production of gelatin. It has been recognized by all the recent workers with actinomycetes that not much emphasis should be laid upon the growth of these organisms on organic media, but that rather synthetic media should be used, which are standard in composition and produce a more characteristic growth. But, by using synthetic media alone, necessarily one has to lay the greatest stress upon the cultural characters and the morphology of the organism. Many difficulties are encountered when one comes to study the morphology of the actinomycetes, which is a task not much simpler than that of the bacteria. It has not even been established as yet that such important characters as club or spiral formation, and size and shape of spores, are constant within a given species, and do not change with the medium upon which the organisms are grown, age of culture, and source of isolation. It is, therefore, our opinion that just as much emphasis should be laid upon the biochemical activities of the organisms as upon their morphology, until the constancy of these characters has been definitely established. Fully recognizing the importance of this fact, in our attempt to classify the different species of actinomycetes, a number of biochemical investigations were conducted, using different proteins and carbohydrates. But these investigations, to be thorough, required a great deal of time, and, since the paper was looked upon by the writers as only preliminary to a series of investigations on the actinomycetes of the soil, only the liquefaction of pure gelatin in distilled water, which is a fairly stable compound, and the pigment production on the gelatin, which seems to be also characteristic of the species, were reported.

#### EXPERIMENTAL

##### *Soils used*

A number of soils collected from different parts of this country have been used for this work. Complete descriptions of the soils, as well as of the fertilization and cropping were published elsewhere (23). These soils will be termed here after the locality from which they were taken. In all, 25 soils were used for the determination of numbers of actinomycetes and bacteria and for the isolation of actinomycetes. These soils are as follows: (1) New Jersey Sassafras garden soil, cultivated; (2) New Jersey Sassafras orchard soil, cultivated; (3) New Jersey clay meadow soil, uncultivated; (4) New Jersey Sassafras forest soil, uncultivated; (5) Iowa Carrington loam, cultivated; (6) Jamesburg cranberry soil from New Jersey; (7) Louisiana sandy loam, culti-

vated; (8) California fertilized orange soil, cultivated; (9) California unfertilized orange soil, cultivated; (10) California upland soil; (11) California adobe soil; (12) California sandy loam; (13) Oregon adobe-like soil, cultivated; (14) Oregon white land, cultivated; (15) Porto Rico clay loam, cultivated; (16) North Dakota wheat soil, cultivated; (17) North Dakota flax soil, cultivated; (18) Hawaiian pineapple soil, cultivated; (19) Alaska soil; (20) Texas Lufkin fine sandy loam, cultivated; (21) Colorado alfalfa soil; (22) Maine Aroostook potato soil, cultivated; (23) Maine Aroostook soil infected with *Spongospora*; (24) Alberta, Canada, soil, cleared in 1909, under grass; (25) Alberta soil, cleared in 1911, cultivated. Soils 1, 2, 3, 10, 11, 12 and 13 are the same as used for the work published before (25).

The samples of all these soils were taken under sterile conditions and shipped in sterile containers. All the samples were plated out as soon as received at this laboratory; this took place during the period between March 1 and May 30, 1916. All soils came in a perfect condition, except the two Dakota and the Colorado soils, which dried out in shipment.

### *Media used*

No special media are required for the bringing out of the numbers of actinomycetes in the soil; they usually develop well on the media used for isolation of soil bacteria. But for the study of the specific characters of the organisms and for the differentiation of the types closely related, special media are necessary.

Two different media have been used for this work:

1. Czapek's solution agar prepared according to the following formula:

Distilled water.....	1,000 cc.
Magnesium sulfate.....	0.5 gm.
Dibasic potassium phosphate.....	1.0 gm.
Potassium chloride.....	0.5 gm.
Ferrous sulfate.....	0.01 gm.
Sodium nitrate.....	2.00 gm.
Saccharose.....	30.00 gm.
Agar.....	15.00 gm.

2. Modified albumen agar. This medium, described in detail elsewhere (23) is made up as follows:

Distilled water.....	1,000 cc.
Dextrose.....	10.00 gm.
K <sub>2</sub> HPO <sub>4</sub> .....	0.50 gm.
MgSO <sub>4</sub> .....	0.20 gm.
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> .....	Trace
Egg-albumen (dissolved in sufficient N/10 NaOH).....	0.15 gm.
Agar.....	15.00 gm.

As soon as the soil samples came to the laboratory, they were thoroughly mixed with a sterile spatula and 10 gm. transferred into 100 cc. of distilled

water. The mixture was shaken five minutes and further dilutions made with sterile tap water. The 10,000 and 100,000 dilutions were plated out in triplicates, on the modified albumen agar as a medium. The plates were incubated at 25°C. and, at the end of three days, the bacteria were counted; the plates were then incubated further to 14 days, when the actinomycetes could be counted. The modified albumen agar, being poor in nutrients, does not allow any rapid-growing organisms, such as molds and certain bacteria, to overgrow the plate. A certain portion of the soil was air-dried and the numbers were figured on the air-dry basis. The numbers present an average of all the plates used.

The data are given in table 1.

TABLE 1

*Numbers of bacteria and actinomycetes in the soil developing on albumen agar*

	SOIL TYPE	BACTERIA		ACTINOMYCETES	
		Numbers	Per cent	Numbers	Per cent
1	New Jersey Sassafras garden.....	5,300,000	85.5	900,000	14.5
2	New Jersey orchard.....	4,800,000	86.6	700,000	13.4
3	New Jersey clay meadow.....	8,100,000	93.7	550,000	6.3
4	New Jersey Sassafras forest.....	610,000	84.7	110,000	15.3
5	Iowa Carrington loam.....	1,764,000	88.2	236,000	11.8
6	Jamesburg Cranberry soil.....	204,500	96.5	7,500	3.5
7	Louisiana sandy loam.....	8,300,000	83.0	1,700,000	17.0
8	California fertilized soil.....	3,570,000	85.0	630,000	15.0
9	California unfertilized soil.....	580,000	63.7	330,000	36.3
10	California upland.....	2,220,000	64.2	1,238,000	35.8
11	California adobe.....	3,620,000	78.0	800,000	22.0
12	California sandy loam.....	6,010,000	80.8	1,430,000	19.2
13	Oregon adobe.....	13,100,000	84.6	2,400,000	15.4
14	Oregon white land.....	3,400,000	91.9	300,000	8.1
15	Porto Rico clay loam.....	2,140,000	69.0	960,000	31.0
16	North Dakota wheat soil.....	2,067,000	68.9	933,000	31.1
17	North Dakota flax soil.....	1,737,000	86.8	263,000	13.2
18	Hawaiian pineapple soil.....	4,334,000	86.7	666,000	13.3
19	Alaska soil.....	6,034,000	79.4	1,566,000	20.6
20	Texas Lufkin fine sandy loam.....	2,126,000	78.7	574,000	21.3
21	Colorado alfalfa soil.....	2,440,000	61.0	1,560,000	39.0
22	Maine Aroostook potato soil.....	4,650,000	94.9	250,000	5.1
23	Maine dark Aroostook infected...	15,900,000	87.8	2,200,000	12.2
24	Alberta grass soil.....	1,110,000	59.4	760,000	40.6
25	Alberta garden soil.....	2,000,000	54.0	1,700,000	46.0
	Average.....	4,245,000	83.0	870,500	17.0

The average of 25 soils taken in different parts of the country gives 4,245,000 bacteria per gram and 870,500 actinomycetes, the latter number representing an average of 17 per cent of the soil flora, exclusive of fungi. There does not seem to be any correlation between the climatic conditions and the number

relationships of the bacteria and actinomycetes. For example, the five most northern soils give different results, the Alaska soil giving 20.6 per cent of actinomycetes, or near the average; the two Maine soils giving 5.1 and 12.2 per cent, which numbers are below the average, while the Alberta soils gave 40.6 and 46 per cent, or much above the average. Among the southern soils we find the Hawaiian with 13.3 per cent, below the average; Porto Rican with 31, almost double the average, and Texas soil, near the average. The fact that the relative numbers of the microorganisms do not depend upon the climatic conditions, but upon the soil type, soil treatment, fertilization and crops grown, is made clear when the soils taken at one locality are compared. The three New Jersey Sassafras soils, under the different treatments, and with widely different numbers of microorganisms, show a similar percentage, somewhat below the average, between the actinomycetes and bacterial numbers, while the fourth soil, the clay meadow, contained only 6.3 per cent of actinomycetes, which is less than a half the proportion in the other three soils.

The two California soils which differ only in fertilization, with larger numbers of microorganisms for the fertilized soil, have shown more than twice as many actinomycetes in proportion to the bacterial numbers in favor of the unfertilized soil, the latter containing 36.3 and the first only 15 per cent of actinomycetes. Of the two Maine soils, a much higher percentage of actinomycetes was found in the infected, than in the normal soil. Great differences in the percentages of actinomycetes and bacteria are also found in the two Oregon and the two Dakota soils. It is interesting to note that the two Alberta soils, one of which was for the four years under grass and the other cultivated, gave 40.6 per cent of actinomycetes for the first soil and 46 per cent for the second one.

On the whole, we find that the heavy soils and those rich in undecomposed organic substances contain large percentages of actinomycetes. Such are the two Alberta soils, which were only a few years under treatment, the Colorado alfalfa soil, the North Dakota wheat soil and the Porto Rico clay loam. Of the three New Jersey Sassafras soils, the forest soil, although unfertilized and of a more acid reaction, which is unfavorable to the growth of actinomycetes, had the highest percentage of these organisms. This can doubtless be explained by the higher content of undecomposed organic substances. These observations bear out the ideas of Hiltner and Störmer (13), Fousek (8), and Conn (3). The differences between the California soils cannot be readily explained because of the insufficient information about those soils. The cranberry soil contained only 3.5 per cent of actinomycetes. This is easily understood, since this soil is under water a large part of the year, and the actinomycetes require good aeration for their development; secondly, this soil is rather acid, which, as will be pointed out elsewhere, is more or less inhibitive to the development of actinomycetes.

*The isolation of types*

Thirty species of Actinomycetes were described by the writers in the first paper (25). Several of these organisms were thought to be the same as were isolated by Krainsky (14). As the comparison was made from descriptions only, no living cultures of Krainsky's organisms being at hand, the identification cannot be complete. Since the publication of the first article, the organisms have been kept in culture, and studied in different media. The further work brought out many more data, which will be presented at a later date. Over six hundred isolations of actinomycetes were made, many species having been isolated from several of the soils. Altogether about 110 distinct strains of actinomycetes were obtained, and this involved a large amount of work. The tendency was not to establish new species for those organisms that may vary slightly from others, but to form groups of organisms which possess common properties, although differing in certain details. In many cases groups were found to run into one another, so that it was often hard to tell whether a certain organism should be placed with one group or with another. The largest and the most troublesome group to work with is the *chromogenus* group, about 15 representatives of which were obtained. Great care has to be exercised in the study of the actinomycetes, and the *chromogenus* group in particular, because organisms that look at first very distinct may prove on further study to be identical; on the other hand, organisms that are found at first to be closely related, may prove on further study to be quite distinct. Such mistakes are made very often, and the authors did not escape them. *Actinomyces violaceus-Caesari*, *A. violaceus-niger* and No. 45, classed at first with the *chromogenus* group, have proved on detailed study to be identical, so that this organism will be spoken of further as *A. violaceus-Caesari*. Further study of the *A. Lipmanii* and the *A. aureus* that were thought in the beginning to be groups of organisms rather than single species, proved that the original proposition was true.

A word should be said concerning the growth of actinomycetes on artificial media. Certain species become well adapted to artificial media and give an earlier and better development after several transfers; others degenerate or die entirely, especially when they are grown on the same medium. When the organisms have been transferred to liquid cultures, soil, or a cellulose medium and then retransferred to Czapek's agar, many of them were thus rejuvenated.

Attention must be called to the fact that the actinomycetes studied by us until now and reported in these papers were isolated from the soil with the use of only egg-albumen agar. Had other media been used for isolation, many other organisms would have doubtless been obtained. This was proven by the fact that several organisms refused to develop on Czapek's agar, while they grew well on gelatin and ammonium sulfate-cellulose media. They probably preferred gelatin and ammonium sulfate to sodium nitrate

**TABLE 2**  
*Distribution of acinomycetes in the soils studied*

[illegible]

as a source of nitrogen. A detailed study of the nutrition of these organisms will be given elsewhere (24). Czapek's medium, used almost exclusively in the previous work (25) for the description of the actinomycetes, is very well suited for this purpose. It is a rather poor medium for the development of actinomycetes; first, because most actinomycetes do not produce invertase so that the saccharose cannot be used rapidly; second, the sodium nitrate is not as good a source of nitrogen as certain organic nitrogen compounds. But, just on account of these deficiencies, this medium allows a very good differentiation of the organisms as to their growth, pigmentation and particularly the production of aerial mycelium.

The distribution of the different species and groups of actinomycetes in the different soils studied are given in table 2.

As seen from table 2, the *A. chromogenus* group, *A. Lipmanii*, *A. aureus*, *A. Rutgersensis*, and the others to a lesser extent, are found in soils collected from different parts of North America and Hawaiian Islands, so that these organisms can truly be looked upon as characteristic of the soil.

#### SUMMARY

1. The numbers of actinomycetes and their relation to bacterial numbers in 25 soils of North America and the Hawaiian Islands were studied.
2. Heavy soils and those rich in undecomposed organic substances are, as a rule, relatively richer in actinomycetes than corresponding lighter soils or soils poor in undecomposed organic matter.
3. The average number of actinomycetes to the total flora of bacteria and actinomycetes, taking an average of 25 soils, was 17 per cent.
4. A cranberry soil which was acid and covered with water part of the time contained only 3.5 per cent of actinomycetes.
5. Many Actinomyces species were isolated from different soils of North America and the Hawaiian Islands, showing their general distribution.

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## THE ORGANIC PHOSPHORUS OF SOIL

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Sometime ago there was published from this laboratory (12) a method for the determination of organic phosphorus in the weak alkali extract of soil. Briefly, this method, which is a combination of the Forbes (5) magnesia mixture and the Emmet-Grindly (4) neutral ammonium molybdate methods, involves first the clarification of an ammonia extract of the soil with a high speed centrifuge; second, precipitation of the resultant clear solution with magnesia mixture; third, the extraction of the well washed precipitate with nitric acid; fourth, the precipitation of this extract with neutral ammonium molybdate; and finally, the determination of the phosphorus in this precipitate. This gives the total inorganic phosphorus in the ammonia extract, which subtracted from the total phosphorus contained therein, gives the phosphorus in organic combination. This method and the results obtained by it have recently been called into question by Gortner and Shaw (7). Their conclusion bearing upon this is as follows:

Probably the greater part of the phosphoric acid present in humus ash is inorganic in nature, being derived from colloidal clay and from phosphoric acid absorbed by the colloids present in the ammonia solution. No method of analysis has as yet been proposed which will distinguish between such absorbed  $P_2O_5$  and organic  $P_2O_5$ .

The purpose of the work herein reported was to test the method further to determine, if possible, whether it actually differentiates between the organic and inorganic phosphorus, in soil. An attempt was made also to discover something of the nature of the organic phosphorus. What success we have had in this two-fold purpose will appear in the following pages.

It is difficult to summarize briefly the data Gortner and Shaw present, which leads them to the conclusion given above. The humus, as they prepared it, contained large amounts of ash, the eight mineral soils which were used showing from 6.7 per cent to 32.6 per cent. The method of preparation of the humus was as follows (6): 15 gm. of air-dry soil with or without previous extraction with 1 per cent acid was shaken continuously for one week with 750 cc. of 4 per cent ammonia. At the end of that time, the solution was decanted from the soil and successively clarified by the addition—first, of  $1\frac{1}{2}$  per cent of potassium sulfate and then of about 2 per cent of ammonium carbonate. After filtering, the solution was evaporated and the humus determined in the

usual way. It will be apparent that not only, as Gortner points out, will high results be obtained for humus because of the long-continued extraction, but also that excessively large amounts of inorganic phosphorus will be dissolved from the soils, large amounts of clay will go into suspension, and finally considerable amounts of inorganic phosphorus will be hydrolysed from any organic compounds present containing that element. Görtner and Shaw believe that because of the high ash content of the humus and because of the high content of phosphoric acid in the humus ash (2.83 to 28.25 per cent) most of it must be in inorganic combination. We will grant that much of the phosphorus in the humus as they prepared it might be in inorganic form, but the same does not necessarily hold true when our method of procedure is followed. The reason for this will be given later.

Further data obtained by them are taken to indicate the non-existence of phosphorus in organic combination. The data show no consistent relation between the amount of phosphorus extracted from air-dry soil and from acid extracted soil and that extracted by 1 per cent acid. This, we believe, proves nothing more than that varying amounts of inorganic phosphorus, both soluble and colloidal, are extracted by the different methods, which, added to a relatively constant amount of organic in the ammonia extracts, leads to the divergent results. Another fact given considerable weight by Gortner and Shaw is that 11.19 per cent of  $P_2O_5$  was found in the humus ash from a subsoil containing only 16 per cent as much humus as its surface soil, whose humus ash contained 18.26 per cent of  $P_2O_5$ . We confess that we fail to follow their argument here, nor do we when they state that 11.19 per cent of  $P_2O_5$  in the humus ash of the subsoil is largely inorganic in nature "due probably to the phenomenon of selective absorption." It no doubt is largely inorganic in nature but it would be simpler and it appears to us more correct to say that it was simply dissolved from the soil. An analogy is drawn between the way the soil acts and the action of Lloyd's reagent (11). This latter material is a hydrous aluminum silicate which absorbs alkaloids completely from acid solutions but gives them up when the reaction is alkaline. It is suggested that possibly soil acts in the same way in regard to phosphoric acid. They then state that if that were true "all of the absorbed phosphoric acid would appear in the humus ash as humus-phosphoric acid and would be counted as organic phosphoric acid in Potter and Benton's method." The analogy of the critics of our method is so forced that little need be said in regard to it. Why should the material after extraction from the soil with ammonia be a perfect absorbent for phosphoric acid in acid solution when the soil alone is not? That soil alone is not, is born out by the fact that acid extracts large amounts of phosphoric acid from soil. Russel and Prescott (13) have shown that dilute acids extract large proportions of phosphorus which has been added to soil as sodium phosphate. They also show, and this may be pertinent, that the soil retained its absorptive power for phosphoric acid after extraction of the humus with 2 per cent sodium hydroxide. At this point it will be well to consider in

more detail the conditions under which the organic phosphorus was determined by our method.

In our work already published (12), one part of soil was shaken with 4 parts of 1.5 or 2 per cent ammonia for 1 to 2 hours. After settling for a few minutes the supernatant liquid was centrifugated in a machine whose bowl was  $4\frac{1}{2}$  inches in diameter. The bowl was run at a speed of 10,000 revolutions per minute. Since the publication of that work a machine whose bowl has a diameter of 2 inches and a speed of 30,000 revolutions per minute has been used. Since centrifugal force varies directly as the square of the speed and directly as the diameter, the centrifugal force of the new machine is about four times that of the old. In the original description (15) of the old machine, results are given showing an average of about 1.4 per cent humus ash in humus from loams and silt loams and 2.2 per cent in clay loams. We have confirmed these results in a general way and in the analysis of a typical Carrington silt loam rather high in organic matter an ammonia extract from 100 gm. of the soil obtained by the old centrifuge gave 0.0414 gm. of ash while the same amount of extract from the new machine gave 0.0243 gm. of ash. This is the only ash analysis we have made with the new machine but enough has been said to make it apparent that analogies should not be drawn from Gortner and Shaw's determinations on humus as they prepared it and from the results from this laboratory. These writers also entirely overlooked an experiment reported in our first paper where inorganic phosphorus was added to the ammoniacal extract of a soil. The extract was then subjected to the regular procedure for the analysis of inorganic phosphorus and a complete recovery of the added phosphorus was obtained. This experiment effectively answers, we believe, the contention that the soluble inorganic phosphorus would be absorbed and the contention that colloidal phosphate-bearing clays make up the "organic" phosphorus is answered by the low ash content of our extracts.

Gortner and Shaw make one more criticism of our paper which must be answered. In taking exception to our statement that 1 per cent hydrochloric acid did not dissolve organic phosphorus from a certain soil they state that if phytin were present calcium and magnesium would be liberated by the acid, thus permitting the free phytin to be dissolved, but our conclusion which was specifically limited to one soil was drawn from an actual experiment. The soil used was very low in organic matter. Since that time we have found that soils high in organic matter may give up 1 or 2 per cent of their organic phosphorus to the 1 per cent hydrochloric acid extract. But that little, if any, of this is phytin phosphoric acid is shown by an experiment reported later in this paper. In brief, it may be stated here that phytin, in quite large amounts, was added to soil and the soil then extracted with 1 per cent acid. No increase due to the phytin was found in the soil. It should be pointed out here that practically all of the known organic phosphorus compounds, with the exception of the lecithins, are appreciably soluble in dilute acids, even nucleic acid, whether in the presence of proteins or not.

Even though the positive experimental proof of the existence of organic phosphorus in soil is questioned, it would seem reasonable to believe that there would be considerable present. Some of the reasons for this belief are as follows. Bacteria are known to contain approximately 1.5 per cent of phosphorus (16), a large part of which is believed to be combined in nucleic acid. While the amount of living bacteria in soil would account for but little of the phosphorus, yet disintegration of the cells would probably not be complete after many generations of the bacteria had died. There is, at present, no way of telling how long it would take for the cells to disintegrate in the soil, but

TABLE 1  
*Phosphorus and carbon content of some Iowa soils*

CLASSIFICATION	COUNTY	POUNDS PER ACRE OF 2,000,000 POUNDS	
		Phosphorus	Carbon
<i>Drift soils:</i>			
Carrington loam.....	Clinton	860	26,960
Carrington fine sandy loam.....	Clinton	740	12,580
Carrington fine sandy loam.....	Clinton	780	10,141
Carrington silt loam.....	Clinton	1,030	45,190
Carrington loam.....	Webster	1,226	66,452
Carrington loam (steep phase).....	Webster	1,020	28,432
Shelby silt loam.....	Scott	1,000	34,160
Shelby loam.....	Scott	800	25,760
Shelby fine sandy loam.....	Scott	480	8,520
Shelby loamy fine sand.....	Scott	400	7,720
<i>Loess soils:</i>			
Memphis silt loam.....	Van Buren	1,095	32,570
Grundy silt loam.....	Van Buren	953	44,220
Muscatine silt loam.....	Scott	1,167	46,573
Lindley silt loam.....	Scott	480	24,240
Wabash silt loam.....	Scott	1,230	40,300
Wabash silty clay loam.....	Scott	1,740	118,960
Sarpy fine sandy loam.....	Scott	1,000	21,460
Sarpy silt loam.....	Scott	2,020	49,100
Sarpy loamy sand.....	Scott	840	17,920

while nucleic acid as a whole is readily attacked by bacteria (16) the pyrimidine half of the nucleic acid molecule is extremely resistant to the action of acid (10) and enzymes (9). Therefore, the continued activity of bacteria in the soil might easily result in an appreciable accumulation of the pyrimidine dinucleotide. On the other hand, of course, there is the opposing action due to the taking up of the organic and soluble inorganic phosphorus as it is formed, or as it is added to the soil, to give insoluble phosphates. But these insoluble phosphates can pass through the cycle of organic phosphorus quite easily, as has been demonstrated from the fact that molds and bacteria readily utilize the phosphorus of quite insoluble phosphates for their life processes.

It is a matter of rather common observation that soils rich in organic matter have a comparatively high phosphorus content. There may be, of course, many exceptions to this, but in general it seems to be true. In table 1 appear a few results not heretofore published which the soil survey department of the station has kindly supplied us. These results are typical of many others which have been secured and are merely selected at random to illustrate the point we are making. The results published in the report of the Soil Survey of Bremer County of Iowa (14) and the various reports of the Soil Survey of the Illinois counties should be consulted in this connection (1). From an examination of these results it is strikingly evident that a high carbon content is usually accompanied by a high phosphorus content. This is certainly indicative, although not absolute proof of the presence of organic phosphorus in soil.

#### EXPERIMENTAL

Gortner and Shaw, it will be recalled, maintained that because ammonia extracted such large and varying amounts of phosphoric acid from acid extracted and non-acid extracted soils, that all of the phosphoric acid so extracted was inorganic. The same conclusion was drawn from the amounts of phosphoric acid extracted from a subsoil when compared with that from its surface soil. In order to gather evidence in regard to those points; the following experiment was carried out.

A sample of surface soil was drawn from humus plot no. 106 and then some of the subsoil, 3 feet below the surface, was taken. Care was taken that the subsoil sample contained no surface soil whatsoever. The sample thus collected was bright yellow in color, and contained practically no organic matter.

No mechanical analysis was made of the soils, but except for the organic matter in the surface soil they were not materially different. The soils were ground to pass a 100-mesh sieve and then part of each sample was extracted with 1 per cent hydrochloric acid. The acid-extracted and non-acid-extracted soils were then subjected to the regular procedure for the determination of organic phosphorus. The results are given in table 2.

It is observed that more organic phosphorus was found in the acid-extracted soil no. 167 than in the non-acid-extracted. This might be due to rendering phytin soluble in ammonia by first dissolving out the readily-soluble bases with the acid. The fact that practically no organic phosphorus was found by our method in the subsoil effectually answers the contention that the "organic phosphorus" is due to clay in colloidal condition.

In order to determine whether phytic acid would be extracted from soil by dilute acid, some barium phytate prepared by the method of Anderson (1) was added to soil which was then extracted with 1 per cent hydrochloric acid. The experiment was conducted as follows:

Two hundred grams of soil were shaken for an hour with 350 cc. of 1.1 per cent hydrochloric acid and 50 cc. of water. Similarly, 200 gm. of soil were

shaken with 1.1 per cent hydrochloric acid and 50 cc. of a very dilute acid solution of the barium phytate. After filtration, 200 cc. of the filtrate was analysed for total phosphorus. The barium phytate contained 16.54 per cent phosphorus and 1.23 per cent of this was inorganic. The results of the experiment are given in table 3.

If all the increase in phosphorus in the acid extract of the phytin-treated soil was due to the inorganic phosphorus in the phytin, only 57 per cent of the total inorganic acid added was extracted. Therefore, it probably is a safe conclusion from this experiment that negligible amounts of phytin are ex-

TABLE 2  
*Phosphorus content of soils*

SOIL	PHOSPHORUS IN THE SOIL					PER CENT ORGANIC PHOSPHORUS OF TOTAL PHOSPHORUS
	Total	In HCl extract	In NH <sub>3</sub> extract	Inorganic in NH <sub>3</sub> extract	Organic in NH <sub>3</sub> extract (by difference)	
	<i>per cent</i>	<i>per cent</i>				
Surface (no. 167).....	0.0490	0.0150	0.0231	0.0041	0.0190	38.7
Surface acid-extracted.....			0.0281	0.0068	0.0213	43.5
Subsoil.....	0.0368	0.0131	0.0091	0.0089	0.0002	0.5
Subsoil acid-extracted.....			0.0085	0.0082	0.0003	0.8
Surface (orchard).....	0.0300	0.009	0.0105	0.0027	0.0078	26.0
Subsoil.....	0.0268	0.009	0.0096	0.0097	None	

TABLE 3  
*Extraction of phytin from soil by dilute acid*

MATERIAL EXTRACTED	PHOSPHORUS ADDED TO SOIL IN PHYTIN		PHOSPHORUS EXTRACTED
	Inorganic	Organic	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Soil no. 167.....			0.00148
Soil no. 167 with Phytin.....	0.00037	0.00495	0.00169

tracted from soil by 1 per cent hydrochloric acid, even when added in an amount about three times the amount of total phosphorus ordinarily dissolved by 1 per cent acid.

#### *Nature of the organic phosphorus of soil*

There have been a few successful attempts to isolate organic phosphorus compounds from the soil but the amounts have been small and the results somewhat uncertain. When it is realized that soil may contain numerous representatives of practically all classes of naturally occurring organic compounds besides soluble and insoluble inorganic compounds, the difficulties en-

countered are not surprising. It was therefore thought that perhaps some information regarding the nature and the availability as plant nutrients of the organic phosphorus of the soil might be gained by a determination of the rate at which organic phosphorus compounds were hydrolyzed by acid. As Jones (10) has recently published results on the hydrolysis of nucleic acid with 5 per cent sulfuric acid at 100°C., it was decided to work under the same conditions.

The work of Jones was first repeated in part, using quite different concentration of the nucleic acid. While there is every reason to believe that the hydrolysis of nucleic acid is a first-order reaction and that, therefore, the percentage hydrolyzed in a given time will be constant, yet it was decided to repeat the work, as it was thought that Jones' conditions might not be duplicated exactly.

Our method of procedure was as follows: Commercial nucleic acid was purified in the usual manner by precipitating three times with glacial acetic acid. After washing in alcohol and ether it was dried in a vacuum, over sul-

TABLE 4  
*Hydrolysis of nucleic acid with 5 per cent sulfuric acid at 100°C.*

DURATION OF HYDROLYSIS	CONCENTRATION: 0.06 GM. PER 50 CC.	JONES' RESULTS: CONCENTRATION 1 GM. PER 20 CC. PER CENT HYDROLYZED
<i>hours</i>		
$\frac{1}{2}$	40.9	29.6
1	49.6	45.6
2	56.8	53.9
3	59.0	55.8
5	62.5	58.5
Total phosphorus .....	7.98	7.94

furic acid and sodium hydroxide. 1.2 gm. of this material was dissolved in the minimum amount of ammonia and then the solution diluted to exactly 500 cc. Then 25-cc. portions, containing 0.06 gm. of nucleic acid, were placed in a series of beakers. In two portions, total phosphorus was determined, in two others inorganic phosphorus, and the remainder were hydrolyzed for different lengths of time as indicated in the table. No inorganic phosphoric acid was found in the unhydrolyzed samples. Sulfuric acid was prepared of such strength that 25-cc. diluted to 50-cc. with water would make the diluted acid exactly 5 per cent; 25-cc. portions of this solution were added to the beakers containing the 25-cc. portions of nucleic acid. These were covered with watch-glasses, and all the beakers placed in an Arnold sterilizer previously brought to the boiling temperature. It took about 10 minutes for the temperature to come back to the boiling point and the time of hydrolysis was taken from the moment the temperature was within 1°C. of the boiling point. It was found by preliminary experiment that the temperature of 50-cc. of water lagged only about two degrees behind that of the sterilizer.



After removal from the sterilizer the solutions were immediately cooled somewhat and neutralized with ammonia, and then precipitated with magnesia mixture and the phosphorus finally determined by the Lorenz method.

The results are given in table 4, and for comparison Jones' results are calculated to the same basis and placed in the table. Jones used more periods of hydrolysis than are given in this table.

It is seen from an inspection of the results that our results are higher than those of Jones. It may be that our conditions were sufficiently different to account for the variation. At any rate, it is apparent that we have to do there

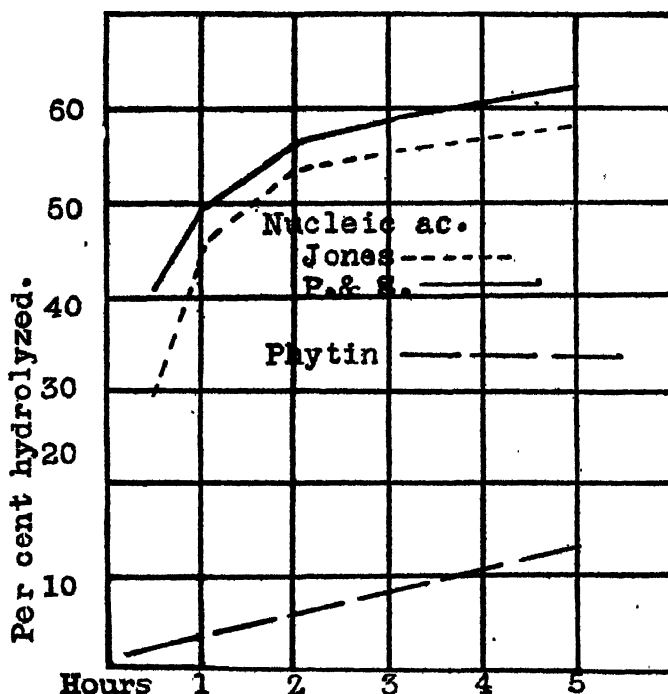


FIG. 1

with a first-order reaction. In figure 1 we have drawn the curves for both sets of results and it is seen that they are quite closely parallel.

An exactly similar experiment was carried out with phytin. The phytin used in this experiment was prepared according to the method of Clark (3) as outlined and modified by Boutwell (2). Because of the spontaneous hydrolysis of phytin the experiment was carried out immediately after preparation of the material. It therefore was not completely anhydrous. A solution of the phytin was prepared so that 25 cc. contained 0.03 gm. of phytin. Analysis of this showed 17.07 per cent of total phosphorus, which, corrected for a small amount of inorganic phosphoric acid found in it gives 16.9 per cent phytin

phosphorus. The results obtained by the hydrolysis experiment on this solution are given in table 5. Also, in this table the results obtained by the 5-hour hydrolysis of approximately twice the concentration of phytin are found. This experiment was made three days later than the other, and, therefore, while twice the weight of phytin was used, yet since it had been kept in a vacuum over phosphorus pentoxide the per cent of phosphorus was somewhat higher, being 18.055, of which 17.97 was found to be phytin phosphorus. In the calculation of the results correction was made for the small amount of inorganic phosphoric acid found in the hydrolyzed phytin.

The results from table 5 have been plotted and appear in figure 1. It is seen that the points fall fairly close to a straight line. Because of this fact and also because nearly the same percentage is hydrolyzed in the 5 hours when the concentration is double, this reaction belongs to the first order. The large divisions on the X axis in the curve for the hydrolysis of phytin make the points

TABLE 5  
*Hydrolysis of phytin with 5 per cent sulfuric acid at 100°C.*

PHYTIN	DURATION OF HYDROLYSIS	PHOSPHORUS HYDROLYSED: PER CENT OF TOTAL PHYTIN PHOSPHORUS
<i>grams</i>	<i>hours</i>	
0.03	0 (blank)	0.788
0.03	$\frac{1}{2}$	2.040
0.03	1	3.050
0.03	2	5.460
0.03	3	8.220
0.03	4	10.070
0.03	5	13.050
0.06	0 (blank)	0.745
0.06	5	12.300

seem unusually close to a straight line, but it was desired to use the same percentage scale here as for nucleic acid.

In figure 2 it is observed that a curve appears for a "dinucleotide." Jones (10) states that all of the phosphoric acid is hydrolyzed from the purine half of the nucleic acid molecule in the first two hours, and therefore after two hours the curve is entirely due to the pyrimidine nucleotides. The results obtained by us on the hydrolysis of nucleic acid have been recalculated on this basis, the results of that calculation gives the curve mentioned above. It simply is the curve which one should obtain after the second hour if the pyrimidine dinucleotide has been started with.

In order to determine whether the organic phosphorus of the soil was hydrolyzed at rates at all analogous to the hydrolysis of these compounds of known constitution, experiments with several soils were planned. The following soils were used:

No. 1. Carrington silt loam. This soil was taken from no. 107 of the humus plots. It has been fallowed for ten years and has received no addition of any organic or other fertilizer materials.

No. 2. Miami silt loam I. This soil was taken from a station orchard. It is considerably lower in organic matter than soil no. 1.

No. 3. Miami silt loam II. Taken from a different place in the same orchard as the other Miami soil, and is somewhat lighter in color and lower in organic matter.

The method followed was to extract the soils with 1 per cent hydrochloric acid and then to determine the total and inorganic phosphoric acid content in

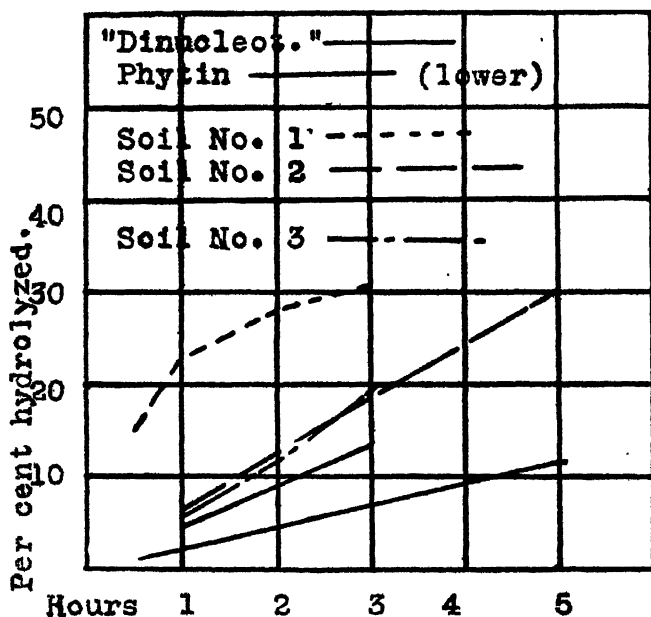


FIG. 2

the ammonia extract. One hundred cubic centimeters of a 1 to 4 extract was used in each case. Then other 100-cc. portions of the extracts were subjected to hydrolysis at 100°C., precisely as has been done in the case of the phytin and the nucleic acid. The results appear in table 6.

The results for the three soils have been plotted and appear in figure 2. As is observed the adjacent points for each soil have been joined by straight lines, as it was not thought that the accuracy of the data warranted the drawing of average curves.

Perhaps the most interesting thing to be noted on an inspection of the curves is the difference between the curve for soil no. 1 and soils no. 2 and 3. The curve for soil no. 1 more nearly resembles the nucleic acid curve, while the curves

for the soils no. 2 and 3 resemble the nucleotide or the phytin curves. Soil no. 1 is naturally higher in organic matter and more fertile, and hence bacterial activity is more vigorous, which perhaps accounts for this. All curves come close enough to the curves for phytin and "pyrimidine dinucleotides" to be perhaps significant, but, of course, from the meager data no definite conclusions can be drawn. While acid hydrolysis cannot be taken as an absolute criterion of availability yet it probably gives comparative results. If so, the organic phosphorus in the two orchard soils is rather unavailable compared with that of the Carrington soil. The results, we believe, indicate quite strongly that in none of these soils do we have much of the tetranucleotide, nucleic acid. The nucleic acid, if present at all, is probably mainly present as pyrimidine nucleo-

TABLE 6  
*Hydrolysis of organic phosphorus in soils with 5 per cent sulfuric acid at 100°C.*

SOIL	TOTAL PHOSPHORUS (PER CENT OF SOIL)	DURATION OF HYDROLYSIS	ORGANIC PHOSPHORUS (PER CENT OF TOTAL PHOSPHORUS)	PER CENT HYDROLYZED OF ORIGINAL ORGANIC PHOSPHORUS
		<i>hours</i>		
No. 1	0.0490		43.5	
		$\frac{1}{2}$	37.0	15.00
		1	33.5	23.00
		2	31.4	27.80
		3	30.1	30.80
No. 2	0.0300		23.1	
		1	21.5	6.80
		2	20.1	13.00
		5	16.2	29.90
No. 3	0.0324		19.8	
		1	18.6	6.06
		2	17.5	11.50
		3	15.9	19.70

tides. This, of course, is not surprising, considering the quite highly-resistant character of this class of compounds. Phytin, also, while very readily attacked by enzymes which are usually associated with it, is relatively resistant to acid hydrolysis, and while it probably would be attacked by bacteria to some extent, yet the vigorous absorptive power of the soil for phytin as compared with the absorption of phosphoric acid as demonstrated by us, probably would indicate that the bacteria would by preference utilize the phosphorus of inorganic phosphoric acid.

#### SUMMARY

1. Gortner and Shaw's conclusion from their results does not necessarily hold for the results obtained by the use of our method for the differentiation of organic from inorganic phosphoric acid in the soil.

2. No "organic phosphorus" was found in subsoil containing no organic matter, but containing considerable amounts of colloidal clay. This proves that "organic phosphorus" is not due to colloidal clay, as is held by Gortner and Shaw.

3. Phytin, when added to soil, is not extracted to an appreciable extent with 1 per cent hydrochloric acid.

4. The hydrolysis curves for phytin and nucleic acid were found, the hydrolysis being effected with 5 per cent sulfuric acid at 100°C. The curve for phytin is a straight line and after 2 hours the curve for nucleic acid is approximately a straight line, thus proving both reactions to be of the first order.

5. The curves for the hydrolysis of the organic phosphorus of three soils were determined. Nothing definite could be drawn from the results, except that nucleic acid as such was evidently not present except in soil no. 1 and then to only a slight extent. The directions of the curves was such that the organic phosphorus might have been due to phytin or a pyrimidine nucleotide.

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See especially the Report of Tazewell County Soils, Illinois (8).

# THE EFFECT OF CERTAIN ORGANIC SUBSTANCES ON SEED GERMINATION<sup>1</sup>

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In a former publication it was reported that the addition of green manures to the soil produces unfavorable conditions for the growth of certain seedlings (3). It seems that the injurious factor, probably soil fungi, develops so rapidly that the young seedlings are killed before they appear above the surface of the soil. This is especially true in the case of seeds rich in oil. Fortunately, the majority of seeds, e.g., the cereals, corn, wheat and oats, are very resistant to the attack of the harmful fungi.

During the progress of this work with green manures data were collected concerning the influence of various organic compounds on germination. The more important results are reported at this time.

The facts presented in the earlier report show that the green manure, clover for example, favors the growth of injurious fungi. If food supply is the controlling factor in the development of the harmful fungi, then it seems probable that other forms of readily available organic matter should produce a similar effect. Accordingly experiments were made using various substances especially adapted to the growth of soil organisms.

## EXPERIMENTAL WORK

The soil used in this work was the Miami silt loam taken from the Station farm. Two kilos of soil were placed in small glazed jars. All of the organic substances were added in dry form to the soil and thoroughly mixed with it. The moisture content was held at half saturation and the temperature was kept at 20°C. Each week after planting, the number of seedlings was counted and the percentage of germination recorded.

Four different substances, alfalfa, casein, peptone, and sugar, were tried in varying amounts. The following seeds were tested: alfalfa, buckwheat, castor bean, red clover, corn, cotton, flax, hemp, white lupine, mustard, oats, serradella, soybean, sunflower, sweet pea, and wheat. According to chemical analysis the seeds included in the preceding group vary greatly in composition. For example, wheat contains less than 2 per cent of fat, while

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castor beans contain more than 50 per cent of fat. The effect of composition of the seed on the injury produced will be noted from the experiments. All results reported in this paper represent the average of at least two separate tests, in many cases more. Except in the case of the small seeds, alfalfa, clover, flax, and mustard, 10 to 20 seeds were planted to each jar; for the small seeds 20 to 50.

### *Nitrogenous substances*

The injury to germination resulting from applications of easily decomposed organic substances may be produced in two or more ways: (a) accumulation of poisonous by-products; (b) increased growth of harmful organisms. It is the aim of this paper to consider especially the biological effect of organic substances on seed germination.

In the first series of tests it was arranged to measure the effect of dry, finely powdered alfalfa, casein, and peptone on the germination of cotton seed. If the increased growth of the harmful fungi in green manured soils is due to the presence of an easily decomposed nitrogenous compound, it seems possible that alfalfa, casein, or peptone may produce a somewhat similar effect. Casein and peptone are especially suitable for the growth of micro-organisms. The effect of these substances on germinating seed is shown by the figures of table 1. Here the average percentage of germination after one, two, and three weeks is recorded.

TABLE 1  
*Effect of alfalfa and of casein on the germination of cotton seed*

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	None	90	90	90	100
2	0.14 alfalfa	100	100	100	111
3	0.28 alfalfa	90	90	90	100
4	0.56 alfalfa	100	100	100	111
5	0.04 casein	85	85	85	95
6	0.09 casein	90	95	95	105
7	0.18 casein	100	100	100	111
8	0.35 casein	75	75		83
9	0.70 casein	60	70		77

In order to make the results comparable to those obtained from green manures when turned under, the weight and percentage of nitrogen in a representative crop were used as the basis of all calculations.

The powdered alfalfa containing 7.38 per cent of moisture was added in amounts equivalent to 0.5, 1, and 2 per cent of green tissue. The casein was added in amounts equivalent to 1, 2, 4, 8, and 16 per cent of green clover, assuming that clover contains 4.4 per cent of protein.

From the data presented in table 1 it is very clear that neither alfalfa nor casein caused any great decrease in the germination of cotton seed. It is true that in certain cases where casein was added in amounts equivalent to 8 and 16 per cent (nitrogen basis) of green clover, there was a decrease in germination. Just why the air-dry powdered alfalfa does not cause a decrease in germination similar to that produced by green alfalfa cannot be explained unless it is due to a change in chemical composition, or to a change in the flora on the alfalfa, or to both factors. Since small amounts of alfalfa or casein produced no harmful effect on germination, it was arranged to make a new test using larger amounts of the nitrogenous substances. For this purpose, powdered alfalfa, casein, and peptone were chosen. The results of the experiment are shown in table 2.

TABLE 2  
*Effect of nitrogenous substances on the germination of cotton seed*

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	None	30.0	70.0	70.0	100
2	0.5 alfalfa	17.0	72.5	72.5	103
3	0.5 casein	7.5	62.5	62.5	89
4	1.0 casein	0	12.5	12.5	18
5	0.5 peptone	12.5	82.5	82.5	118
6	1.0 peptone	5.0	20.0	20.0	28

Here again, the data of the table failed to show any decrease in germination when dry powdered alfalfa, casein, or peptone were applied in small quantities. When added in larger quantities, more than one-half of 1 per cent, there was a decided decrease in germination. From the figures of these two tables it is plain that nitrogenous compounds such as alfalfa, casein and peptone decomposing in the soil, do not, unless they occur in large amounts, cause any change in the germination of cotton seed. The conclusion seems justified that the influence on germination of dry powdered alfalfa, casein, and peptone is very different from that of green clover or green alfalfa. In this connection the question of reaction suggests itself. Accordingly experiments were planned to see what effect casein and alfalfa powder, in amounts large enough to produce a serious injury, would have on the germination of soybeans in the presence of calcium carbonate. According to von Brehmer (1) lime increases the percentage of germination. After mixing the nitrogen compounds thoroughly with the soil, 10 soybean seeds were sown in each jar. The average percentage of germination is shown in table 3.

Unlike the results of previous tests, 0.5 per cent of casein decreased the percentage of germination, while 1 per cent of casein entirely prohibited germination. The addition of carbonate of lime with the casein failed to



TABLE 3

*Effect of nitrogenous substances and calcium carbonate on the germination of soybeans*

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	None	65	75	75	100
2	0.5 casein	50	50	50	66
3	1.0 casein	50	0	0	0
4	1.0 casein and 1 CaCO <sub>3</sub>	20	0	0	0
5	0.5 alfalfa	45	85	85	113
6	0.5 alfalfa and 1 CaCO <sub>3</sub>	45	70	75	100
7	1.0 alfalfa	45	55	55	74
8	1.0 alfalfa and 1 CaCO <sub>3</sub>	50	70	70	93
9	2.0 alfalfa	30	35	35	46
10	2.0 alfalfa and 1 CaCO <sub>3</sub>	40	50	50	66

overcome the harmful effect on germination. Plate 1, figure 1 is the photograph of the number of seedlings two weeks after treatment.

Where alfalfa powder alone was added to the soil, it required more than 0.5 per cent to retard germination. Although the evidence is not conclusive, it seems that limestone tends to overcome in part the injury caused by alfalfa. This difference in germination of soybeans in the treated and untreated soil is shown in plate 1, figures 2 and 3.

#### *Nitrogen-free substances*

It was found that when nitrogenous substances, i.e., alfalfa, casein, and peptone are added to the soil in quantities equivalent to the nitrogen present in an average crop of green alfalfa, normal germination occurred. There seems to be no doubt that the dry nitrogenous substances must be added in much larger amounts in order to injure germination seriously. Although the data are limited, it is reasonably safe to conclude that the presence of small amounts of these readily available nitrogenous compounds fails to bring about the proper conditions for the development of the harmful fungi. Accordingly, it was planned to measure the effect of certain carbohydrate compounds on germination. Cane sugar, or saccharose, was employed because this substance offers a very suitable carbohydrate compound for the majority of microorganisms.

In a former paper it was found that decomposing green plant tissue favors the growth of fungi which are destructive to seedlings. If the damage to the germinating seeds is due to the addition of food for the fungi, it seems probable that a soluble carbohydrate, e.g., saccharose, should produce a somewhat similar effect. It has been shown repeatedly that the addition of cane sugar to soil is followed by an enormous increase in the number of bacteria and a parallel increase in carbon dioxide evolution. The great gain in carbon

dioxide will no doubt prove detrimental to seed germination (7, 17). The references given below illustrate this point. Engberding (2) found that 2 per cent sugar (saccharose) caused an increase in the number of bacteria from about 1000 to 1500 per cent. Miller (10) obtained the following results from the use of 2 per cent of dextrose in Goettingen soil:

*Number of bacteria per gram of soil*

	AFTER		
	8 days	21 days	41 days
Control.....	9,100,000	14,800,000	17,400,000
Sugar, 2 per cent.....	74,800,000	189,500,000	393,200,000

The enormous gain in number is soon followed by a rapid decrease.

In order to find out the effect of sugar and of sterilization on the number of bacteria in Miami soil, a series of counts was made. The soil was placed in small jars, treated as shown below, and incubated at approximately 20°C. in the greenhouse. No precaution was taken to keep the sterilized soil free of bacteria. At regular intervals of two days each unsterilized tap water was added to keep the soil at approximately 18 per cent of moisture. In this way the sterilized soil soon became thickly seeded with bacteria. Plate counts were made two weeks after treatment in the first test and four weeks in the second test.

*Two weeks after treatment*

	<i>Bacteria per gram of dry soil</i>
1. Unsterilized soil.....	8,966,000
2. Sterilized soil.....	61,412,000
3. Sugar, 1.5 per cent.....	292,323,000

*Four weeks after treatment*

1. Unsterilized soil.....	58,081,000
2. Sugar, 1 per cent.....	203,280,000
3. Alfalfa, 0.5 per cent.....	358,422,000

The results are in accord with the reports of others, namely, sterilization alone usually brings about favorable conditions for the multiplication of bacteria. Sugar and alfalfa cause an enormous gain in the number of soil bacteria. Just what effect this increase in the soil flora will have on the germination of seed is shown in the figures of table 4. Here a record was kept of the average percentage germination of mustard seed and the total number of bacteria per gram of dry soil.

No precautions were taken to prevent contamination after the mustard seed were planted. It will be seen from the figures of the table that sugar was injurious to mustard seedlings (4). Moreover, the injury was corrected in part by sterilization, although sterilization alone seems to have produced a condition slightly injurious to germination. The injury to germination

from sterilization of soil has been noted from various sources (6, 14, 15). Plate 2 shows the effect of sterilization on mustard seedlings. The plants in jars 3 and 4 are much behind those of jars 1 and 2. This picture was taken fourteen days after planting.

As regards the number of bacteria it will be found that sterilization alone caused an enormous gain, which is especially noticeable after 21 days. Sugar alone caused a rapid increase in number followed by a decrease after two weeks. Just why the sterilized, sugar-treated soil at this date should con-

TABLE 4

*Effect of sugar on the germination of mustard seed in sterilized and unsterilized soils*

NUMBER	TREATMENT	GERMINATION			BACTERIA PER GRAM OF DRY SOIL	
		2 weeks	3 weeks	Relative	14 days	21 days
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
1	None	100	100	100	12,559,000	14,248,000
2	Sterilized	80	85	85	50,263,000	112,253,000
3	Cane sugar, 1	60	60	60	256,350,000	45,235,000
4	Cane sugar 1 sterilized	60	75	75	4,717,000	61,990,000

TABLE 5

*Effect of varying amounts of sugar on the germination of cotton seed*

NUMBER	TREATMENT	GERMINATION			RELATIVE
		1 week	2 weeks	3 weeks	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	None	42.5	80	85.0	100
2	0.25 sugar	45.0	85	85.0	100
3	0.50 sugar	50.0	85	87.5	103
4	1.00 sugar	32.5	85	87.5	103
5	2.00 sugar	27.5	80	80.0	94
6	3.00 sugar	15.0	75	77.5	91
7	5.00 sugar	0	30	0*	0

\* All seedlings killed by the end of the third week.

tain fewer bacteria than the untreated is not known. From the data it will be seen that the harmful factor is not a result of enormous *increase* in the number of bacteria.

*Effect of different amounts of sugar.* This experiment was prepared by adding to Miami silt loam soil cane sugar in varying amounts from 0.25 to 5 per cent. After planting the soil moisture was increased to one-half the total water-holding capacity. The results of this experiment are presented in table 5.

Here it was found that normal germination occurred in all cases with amounts of sugar from 0.25 to 1 per cent, while greater amounts proved in-

jurious. In the presence of 5 per cent all the seedlings were killed by the third week. The retarding effect of sugar on germination may be explained in part by two factors: first, the rapid evolution of carbon dioxide; second, the formation of acids.

*Effect of sugar on various seeds.* In order to secure additional data concerning the relation of sugar to the process of germination, seeds from the following plants were used: alfalfa, buckwheat, castor bean, corn, flax, hemp, oats, red clover, serradella, soybean, sunflower, sweet pea, and wheat. Throughout this test the same type of soil with 1 per cent of sugar was

TABLE 6  
*Effect of sugar on the germination of various seeds*

NUMBER	SEED	TREATMENT	GERMINATION			RELATIVE
			1 week	2 weeks	4 weeks	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Alfalfa	None	82.5	82.5	82.5	100
2	Alfalfa	1 sugar	65.0	72.5	72.5	88
3	Buckwheat	None	70.0	85.0	87.5	100
4	Buckwheat	1 sugar	75.0	80.0	85.0	97
5	Corn	None	90.0	90.0	90.0	100
6	Corn	1 sugar	30.0	85.0	85.0	94
7	Flax	None	85.0	87.5	90.0	100
8	Flax	1 sugar	40.0	47.5	67.5	75
9	Hemp	None	87.5	87.5	87.5	100
10	Hemp	1 sugar	62.5	82.5	82.5	94
11	Oats	None	90.0	92.5	95.0	100
12	Oats	1 sugar	87.5	97.5	97.5	103
13	Red clover	None	55.0	62.5	62.5	100
14	Red clover	1 sugar	62.5	62.5	70.0	112
15	Serradella	None	62.5	62.5	62.5	100
16	Serradella	1 sugar	32.5	42.5	42.5	68
17	Sunflower	None	100.0	100.0	100.0	100
18	Sunflower	1 sugar	100.0	100.0	100.0	100
19	Sweet pea	None		72.5	72.5	100
20	Sweet pea	1 sugar		77.5	77.5	107
21	Wheat	None	87.5	90.0	90.0	100
22	Wheat	1 sugar	80.0	100.0	100.0	111

employed. The results of the tests are given in table 6. Because of poor germination, the results with the castor beans were omitted from the table. The figures of the table show very clearly the retarding effect of sugar on seed germination. Apparently the young seedlings soon recover from the period of depression, for within 4 weeks after treatment the percentage of germination in the presence of sugar is nearly as high as in the control. In a few cases the number of seedlings in the sugar-treated series was equally as great or exceeded that of the control. Aside from the effect on germination, the sugar-treated soils were characterized by an acid reaction, and apparently

the physical properties of the soil were changed, causing the soil to become very compact.

An illustration of the effect of sugar on seedlings is given in plate 3, figures 1 to 3, and in plate 4, figures 1 to 3.

Although no consistent decrease in percentage of germination was noted, there was a decided decrease in plant growth. The sugar-treated plants were far behind the untreated; they were not only small, but were also marked by a yellow color. Probably the pale color and abnormal development of plants in sugar-treated soils is partly a result of the lack of available nitrogen (11, 12, 16). The great increase in the number of bacteria in the treated soil is no doubt followed by a decrease in soluble nitrogen.

The results of germination tests with corn are in accord with the reports of earlier investigators. Mazé (9) and Lipman (8) noted that sugar retarded

TABLE 7

*Effect of cane sugar on germination of flax, mustard, lupine, and sunflower seed*

NUMBER	SEED	TREATMENT	GERMINATION		RELATIVE
			1 week	2 weeks	
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	Flax	None	85.0	85	100
2	Flax	1.5 sugar	45.0	65	76
3	Mustard	None	95.0	95	100
4	Mustard	1.5 sugar	42.5	65	68
5	White lupine	None	90.0	90	100
6	White lupine	1.5 sugar	90.0	90	100
7	Sunflower	None	50.0	65	100
8	Sunflower	1.5 sugar	25.0	55	84

both the germination and the growth of corn. A somewhat similar effect was noted by Pfeiffer (13) concerning the action of sugar on oats.

Additional data in regard to the effect of sugar on germination or on very young seedlings is shown in table 7. This test represents a study of the relation of certain oil seeds to sugar.

The influence of the sugar is well marked at the end of the second week. Flax and mustard seem especially sensitive to this treatment.

*Effect of sugar and limestone.* If the retarding effect of sugar on germination is due to acidity, then the presence of a base should tend to overcome this condition. Although repeated tests were made using different quantities of lime, no decided effect on the germination of soybeans was noted.

*Effect of sugar and lime in sterilized soil.* Here three soil types, Miami silt loam, sand, and a mixture of equal parts of these two soils, were used in duplicate. It was thought that perhaps a change in the physical, as well as in the chemical properties of soil, would influence the rate of germination. For example, the products of decomposition differ in a compact soil with a

limited supply of oxygen from those in an open type of soil. Calcium oxide was used in place of calcium carbonate. The average percentage germination is given in table 8.

From the data it may be seen that in unsterilized soil sugar retarded the rate of germination. Probably the carbon dioxide formed in the decomposition of sugar was the cause. As shown from previous results, most probably the percentage of germination in the presence of sugar would have been much greater if the experiment had been continued for a week or two longer. The effect of sugar is noted by the decrease in number and size of seedlings. Unlike the seed in green manured soil, examinations after two weeks showed that many of the seeds were just beginning to sprout. The sugar *retarded* germination but did not cause the seed to *decay*.

TABLE 8

*Effect of sugar and lime on the germination of cotton seed*

NUMBER	TREATMENT	GERMINATION AFTER 2 WEEKS		
		Clay soil	Sandy soil	Sandy clay soil
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	None	90	30	90
2	Sterilized	100	90	80
3	0.2 CaO	90	70	80
4	0.2 CaO, sterilized	90	90	90
5	1.5 sugar	60	30	60
6	1.5 sugar, sterilized	90	90	90
7	1.5 sugar } 0.2 CaO }	60	50	70
8	1.5 sugar, sterilized } 0.2 CaO }	95	90	90

#### SUMMARY

1. Nitrogenous substances such as alfalfa powder, casein, and peptone do not seriously injure seed germination unless used in very large quantities.

2. As compared with green manure (nitrogen content), very large amounts of casein and peptone are required to cause a noticeable decrease in germination.

3. Calcium carbonate apparently does not lessen the decrease in germination due to very large applications of alfalfa powder or casein.

4. Sugar greatly increases bacterial growth and retards the rate of seed germination. In large amounts it decreases the percentage of germination.

5. The retarding action of sugar on the germination of seeds is perhaps due to the large amount of carbon dioxide given off in the decomposition of the sugar.

6. Soil sterilization often inhibits the rate of seed germination.

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## PLATE 1

## EFFECT OF CASEIN AND ALFALFA ON GERMINATION OF SOYBEANS

Fig. 1. Jars 1 and 2 untreated; 3 and 4 received 0.5 per cent casein; 5 and 6 received 1.0 per cent casein, and 7 and 8 received 1.0 per cent casein and 1.0 per cent limestone.

Fig. 2. Jars 1 and 2 untreated; 3 and 4 received 0.5 per cent dry alfalfa hay; 5 and 6 received 1.0 per cent dry alfalfa hay, and 7 and 8 received 2.0 per cent dry alfalfa hay.

Fig. 3. The jars in this series received the same treatment as those in figure 2 plus 1.0 per cent limestone.

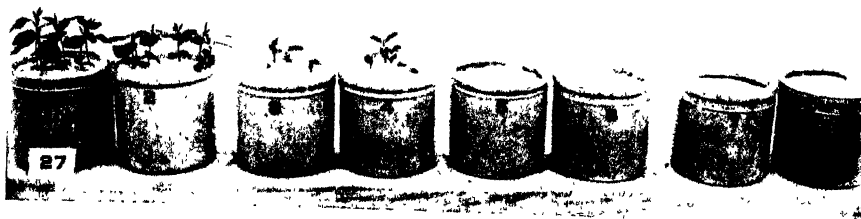


FIG. 1



FIG. 2



FIG. 3



## PLATE 2

### EFFECT OF STERILIZING SOIL ON MUSTARD GERMINATION

The soil in jars 1 and 2 was untreated; in jars 3 and 4 it was sterilized for 3 hours in an autoclave.



### PLATE 3

#### EFFECT OF SUGAR ON GERMINATION

Jars 1 and 2 untreated, 3 and 4 received 1 per cent cane sugar.

Fig. 1. Corn

Fig. 2. Oats

Fig. 3. Wheat



FIG. 1

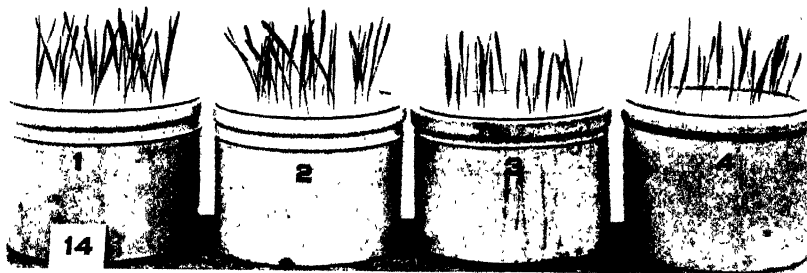


FIG. 2

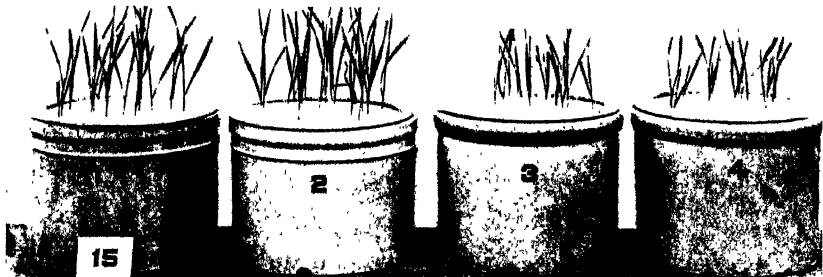


FIG. 3

**PLATE 4**

**EFFECT OF SUGAR ON GERMINATION**

Jars 1 and 2 untreated; 3 and 4 received 1 per cent cane sugar.

Fig. 1. Buckwheat

Fig. 2. Hemp

Fig. 3. Flax



FIG. 1

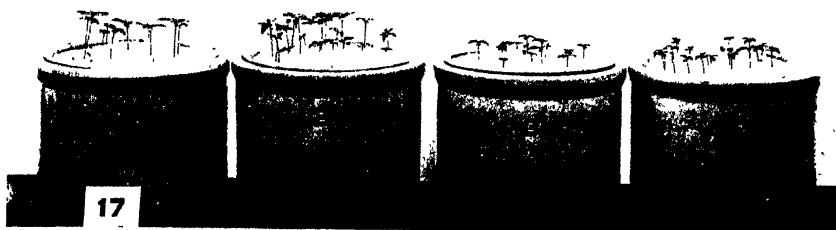


FIG. 2

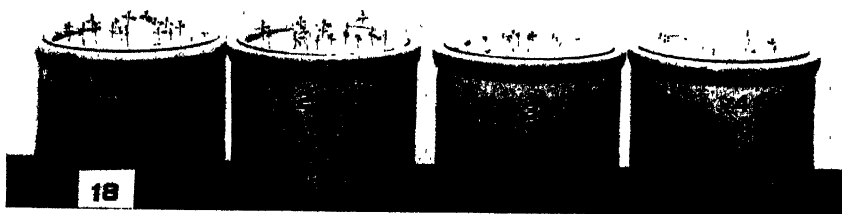


FIG. 3



## EFFECT OF SULFOFICATION AND NITRIFICATION ON ROCK PHOSPHATE<sup>1</sup>

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Recent investigations have directed attention to certain biochemical processes as effective agents for converting the phosphorus of rock phosphate into a more available form. The action of the acidity produced by oxidation of sulfur has been proposed by Lipman and associates (4) as a means for rendering the phosphorus of rock phosphate available when mixed with soil and sulfur in compost heaps. The solvent effect of nitrous acid formed during the course of nitrogen transformations in soil is considered by Hopkins and Whiting (2) to have an important bearing on the question of availability of insoluble phosphates. They state that 115 pounds of phosphorus soluble in water were secured by the oxidation of 56 pounds of nitrogen. The conclusions of the latter investigators are based on experimental data obtained from mixtures of purified calcium phosphate and ammonium sulfate in solution cultures.

On account of the increasing cost of acid phosphate during the past two years, studies of the effects of sulfur oxidation and nitrification on phosphates were undertaken at the Ohio Station in November 1917. That phase of the investigation pertaining to the effect of nitrification on availability of phosphates naturally present in soil or added to it, was taken up in connection with the subject of sulfur oxidation. The investigation was so arranged that the influence of nitrification on phosphates could be determined on the same soil mixtures that were used for studying the effect of sulfur oxidation on phosphates.

The question of whether the method of composting as proposed by Lipman and associates would be practical for Ohio conditions, influenced us to plan our experiments so that the quantity of rock phosphate used would more nearly approach that which is applied in field practice, notwithstanding that the opportunity for action of the acidity on the phosphate under these conditions would be much less than if larger amounts of the materials were intimately mixed together. Rock phosphate and sulfur were therefore used in a preliminary experiment at the rate of 1000 pounds per million of soil. While the results obtained showed that the sulfur was as readily oxidized as if a larger amount had been used, and no difficulty was experienced in measuring

<sup>1</sup> G. E. Boltz and J. A. Stenius have assisted with the analytical work.



the amount oxidized, it was found impossible to determine to what extent the availability of the phosphorus had been changed on account of the adsorption capacity of the soil for phosphorus. It was therefore deemed advisable to use larger amounts than would be practical in the field, in order to obtain indications of interactions which had occurred under different conditions of treatment, and this was done in the experimental work begun in November, 1917.

More recently Kelley (3) has reported the results of an investigation which included a study of the effect of the oxidation of soil nitrogen, and that supplied by dried blood and ammonium sulfate in soil mixtures with and without the addition of calcium carbonate. His procedure for the study of the solubility of phosphate more nearly approximates field conditions than do those experiments where the conclusions were based on results obtained from solution cultures.

The general plan of the experimental work reported in this paper is somewhat similar to that followed by Kelley in his study of the influence of nitrification on solubility of phosphates, in that dried blood and ammonium sulfate were used as sources of nitrogen, and additions of calcium carbonate were made in some instances to determine whether the acidity developed by the processes would have a selective action on the calcium of added phosphate in soils naturally calcareous.

#### EXPERIMENTAL PROCEDURE

A series of 56 mixtures which included various treatments of soil, peat and a pure quartz sand were used as mediums for studying the action of nitrification and sulfification. An additional series of sand cultures were used for a further study of the effect of nitrification on phosphorus, for the reason that in some preliminary work with soils indications had been furnished by the calcium in water solutions of soil treated with rock phosphate and organic nitrogen, that the phosphate had been acted on by some agency, although no increased amount of soluble phosphorus was found. This was considered to be due to the absorptive capacity of the soil for phosphorus.

The soils, which were a silt loam and a black clay, differed considerably in their composition and physical characteristics. The acid silt which was used for the greater number of the experimental mixtures has a requirement for base equivalent to 4000 parts of calcium carbonate per million of soil, and is rather deficient in organic residues; it has a total nitrogen content of 1000 parts per million. The black clay used is naturally basic and contains 4000 parts of nitrogen per million. The phosphorus content of the black clay is about 1000 parts per million and the sulfur 500. These elements are present in smaller amounts in the silt loam, the phosphorus content being 450, and the sulfur 200 parts per million of soil. The soil was air dried and ground finer than 2 mm.

Two forms of nitrogen were used, dried blood and ammonium sulfate. In the mixture when either of these carriers were included as a part of the treatment, 4 gm. of blood and 4 gm. ammonium sulfate were added to 500 gm. portions of soil.

A Tennessee brown rock phosphate analyzing 12 per cent phosphorus furnished the supply of insoluble phosphorus in certain mixtures. When sulfur was included as a part of the treatment the amount added was 2 gm.

Additions of varying amounts of calcium carbonate were made to certain mixtures of the acid silt loam soil in order to study the effect of the processes in an acid soil and in soil supplied with basic material. The amounts of calcium carbonate added to certain mixtures of the silt loam are expressed in the tabulations of results. The silt loam soil has a requirement for calcium carbonate which is slightly less than 4000 pounds per million of soil. The largest addition of calcium carbonate was at the rate of 8000 pounds per million, and decreasing amounts provided for different degrees of basicity.

After the various treatments were thoroughly mixed with the 500 gm. portions of soil, sufficient water was added to satisfy 60 per cent of the water holding capacity, and the mixtures transferred to the quart jars used for containers. Duplicates of all mixtures were prepared. The contents of the uncovered jars were stirred after each addition of water, which was made every fourth day, so that the supply of oxygen necessary for the activities of the organisms was provided. The soil mixtures were incubated at a temperature of 30°C. for a period of 19 weeks from November 21, 1917 to April 15, 1918.

Previous to adding water, portions of the mixtures were withdrawn and citrate soluble phosphorus determined to measure any change in the solubility of phosphorus in rock phosphate and soil, resulting from possible interactions of the materials before biological activities had progressed.

The indications of the effect of sulfur oxidation and nitrogen transformations on availability of phosphorus at the end of the experimental period, April 10, 1918, were obtained from the phosphorus soluble in neutral ammonium citrate solution. This determination was made according to the regular method for available phosphorus in fertilizing materials, the soil mixtures being air dried and thoroughly mixed previous to extracting with the citrate solution.

Measurements of nitrification and the extent to which oxidation of sulfur had proceeded were secured by extracting 400 gm. portions of the mixt res with 2500 cc. of distilled water for 15 hours, with continuous shaking for 3 hours.

Portions of the water extract filtered through Berkfield filters were used for determinations of nitrates, water soluble sulfur and sulfates, and calcium.

Nitric nitrogen was determined by the modified Devarda reduction method (1). The water extracts of the soil mixtures were tested for nitrites, but no appreciable quantities were present, the largest amount found in any

of the mixtures being less than 1 part per million. The relative acidity of the water extracts was determined by titration, using methyl red for the indicator. Calcium was determined volumetrically, and sulfur precipitated as barium sulfate, according to the approved procedures.

#### EFFECT OF SULFUR OXIDATION

A considerable quantity of sulfur in a finely divided condition, mixed with soil at the rate of 4,000 parts per million of soil has been converted into sulfuric acid as indicated by the sulfates in the water solution at the expiration of the incubation period. Approximately 50 per cent of the added sulfur has been oxidized, the amount recovered varying somewhat, depending upon the soil and the materials included as part of the treatment.

Aside from a plentiful supply of oxygen, there are other factors which appear to be necessary for the active development of the sulfur-oxidizing

TABLE 1

*Effect of sulfur oxidation on rock phosphate in sand after 19 weeks incubation (Data expressed as parts per million of dry sand mixtures)*

ADDITIONS TO 500 GM. SAND	CITRATE-SOLUBLE PHOSPHORUS	WATER-SOLUBLE	
		Calcium	Sulfur
	p. p. m.	p. p. m.	p. p. m.
None.....	7	7	30
Sulfur, 2 gm.....	8	33	42
Sulfur, 2 gm.; calcium carbonate 2 gm.....	8	656	503
Rock phosphate, 8 gm.....	214	46	31
Rock phosphate 8 gm.; sulfur, 2 gm.....	242	150	166

organisms. A series of mixtures where sand was used as a medium show the effect of the presence of a basic calcium compound in promoting sulfur oxidation. From the results tabulated in table 1, it will be observed that in a sand medium the presence of calcium carbonate has promoted the oxidation of sulfur, since in the sand mixtures where it was present, the quantity of sulfur oxidized increased from 42 to 503 parts per million which indicates that some neutralizing agent for the acid formed, as calcium carbonate in this case, is essential for the continued activities of the sulfofying organisms. The results in table 2, for sand mixtures containing a smaller amount of sulfur, also show the influence of calcium carbonate. In both these series of mixtures no provision was made for inoculation, but regardless of this, the oxidation of sulfur was quite active when calcium carbonate was included.

Conflicting indications are obtained with respect to the effect of calcium carbonate on sulfonation in sand, and in soil cultures, since the results for soil mixtures which will be discussed later, show that the presence of calcium carbonate has tended to depress sulfonation.

If the results for the sand cultures are considered to furnish the more correct explanation concerning the influence of calcium carbonate on sulfofication, the effect its addition has had can be interpreted as meaning that a basic compound is necessary. The results for sulfur oxidized in the acid soil show that bases other than calcium serve to neutralize the acidity produced, because this soil contains no calcium carbonate, and only a small amount of calcium in other combinations.

The smaller increase in oxidized sulfur which occurred in sand cultures where rock phosphate was used with sulfur in the absence of calcium carbonate, indicates that as a source of base necessary for the activities of the sulfofying organisms, rock phosphate is much less effective than calcium carbonate. This is also apparent from the results for nitrification of blood and ammonium sulfate in acid soil mixtures which included rock phosphate with these nitrogen carriers.

The effect of calcium carbonate on sulfur oxidation in an acid soil is shown in table 3, which gives the data pertaining to the effect of sulfur oxidation on

TABLE 2

*Sulfur oxidation and availability of phosphorus in sand mixtures, after 17 weeks incubation.*  
(Data expressed as parts per million of dry sand mixtures)

ADDITIONS TO 500 GM. SAND	CITRATE-SOLUBLE PHOSPHORUS	WATER-SOLUBLE	
		Calcium	Sulfur
	p. p. m.	p. p. m.	p. p. m.
Rock phosphate, 0.5 gm.....	30	25	0
Rock phosphate 0.5 gm., sulfur, 0.5 gm.....	60	98	70
Rock phosphate 0.5 gm., sulfur 0.5 gm., calcium carbonate 2 gm.....	25	722	591

rock phosphate in soil mixtures. It may be that phosphorus, although converted into a more soluble form, will be fixed by soil to such an extent that a correct measure of changes in its availability, due to the action of acidity resulting from the oxidation of sulfur or other biochemical process, can not be obtained from the phosphorus results alone.

If there is any appreciable reaction between insoluble calcium phosphate and sulfur acidity, with the formation of calcium sulfate, the calcium in the water solution may furnish a more reliable indication than will be given by the figures for phosphorus soluble in neutral ammonium citrate solution. Where rock phosphate and sulfur were in contact in sand mixtures the fixation of phosphorus was no doubt largely, if not altogether, eliminated, and the citrate-soluble phosphorus as well as the calcium should furnish evidence of any appreciable action the sulfur acidity has had on the rock phosphate.

The phosphorus results for the sand mixtures given in table 1 show some increases in the availability of phosphorus at the end of the 19 week period, where rock phosphate and sulfur were used together. A similar result was

also obtained in the group of mixtures where sulfur and rock phosphate were added at the rate of 0.5 gm. to 500 gm. of sand, table 2.

When an acid soil was employed as a medium, the results obtained are of more interest on account of the wider range of treatment. In this soil series, the data for which are given in table 3, the addition of calcium carbonate provided for different degrees of basicity.

It will be noted that the effect of calcium carbonate when added in amount sufficient to satisfy the soil's requirement has been to depress the oxidation of sulfur as indicated by  $\text{SO}_4$  in the water extract. When approximately half of the soils requirement was satisfied the quantity of sulfur oxidized increased,

TABLE 3

*Effect of sulfur oxidation in acid silt loam soil, after 19 weeks incubation. (Data expressed as parts per million of dry soil mixtures)*

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acidity*	Alkalinity*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.
None.....	45	60	111	37	84	0	3
Calcium carbonate, 2 gm.....	48	54	270	50	128	0	14
Sulfur, 2 gm.....	44	99	370	2,143	6	338	0
Sulfur, 2 gm.; calcium carbonate, 4 gm..	53	72	858	1,294	35	4	0
Sulfur, 2 gm.; calcium carbonate, 2 gm..	45	74	785	1,923	21	194	0
Rock phosphate, 8 gm.....	53	98	114	642	105	0	2
Rock phosphate, 8 gm.; Calcium carbonate, 2 gm.....	67	96	280	64	128	0	14
Rock phosphate, 8 gm.; sulfur, 2 gm....	50	630	728	1,900	6	192	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; Calcium carbonate, 4 gm.....	59	120	1,361	1,401	50	6	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; Calcium carbonate, 2 gm.....	59	132	981	1,592	12	75	0

\*Acidity expressed as  $\text{H}_2\text{SO}_4$ , and alkalinity as  $\text{CaCO}_3$

but was less than the amount obtained in solution from the soil mixture to which sulfur only was added.

The quantities of citrate-soluble phosphorus found at the beginning of the experiment in the soil mixtures receiving additions of rock phosphate and sulfur do not differ materially from those in the soil receiving no phosphorus, as would be expected, since no incubation period had intervened between the time the materials were incorporated and the time when samples were withdrawn for measuring any changes which had occurred. At the end of the 19 week period there was a small increase in all of the mixtures, the most pronounced increases, aside from those observed where rock phosphate and sulfur were in contact, were found in the soils receiving additions of rock phosphate and sulfur separately.

The only considerable increase noted was that resulting from oxidation of sulfur incorporated with rock phosphate where no other additions were made. The proportion of rock phosphate to soil was such that phosphorus was added at the rate of 1900 parts per million. The effect of sulfur when incorporated with rock phosphate, in the absence of calcium carbonate or nitrogen carriers, was responsible for the accumulation of 630 parts per million of available phosphorus on the basis of the dry soil.

While the presence of calcium carbonate has depressed the oxidation of sulfur, the quantity of sulfates found indicates that there was sufficient acidity to have attacked the rock phosphate, providing that no calcium carbonate had been present. The results for available phosphorus in the acid soil mixtures, to which additions of calcium carbonate were made, show that the action of oxidized sulfur on rock phosphate will be decreased considerably in soils containing calcium carbonate. Further evidence of this is furnished by the results for the black clay soil which is decidedly basic although its natural calcium carbonate content is only 300 parts per million.

The basic clay used as a medium contains phosphorus in a form readily acted on by the acidity produced by sulfofication, according to the indications given by the results where no rock phosphate was added either alone or in combination with a nitrogen carrier. It will be observed from the results for the basic clay soil, which are given in table 4, that while the oxidation of sulfur has proceeded actively, the quantity of sulfates extracted with water being similar to that from the acid soil, rock phosphate has not been attacked to the same extent. Where rock phosphate and sulfur together were the additions, the citrate soluble phosphorus increased from 129 parts per million at the beginning of the experiment to 479. In all instances where sulfur was in contact with rock phosphate, its oxidation product attacked the phosphate, but the amount of citrate soluble phosphorus was in no case as large as that obtained when sulfur was used alone with rock phosphate in the acid soil. The natural basicity of the black clay furnishes the explanation of this difference. The concentration of calcium in the water extract of the basic soil shows that it is well supplied with soluble calcium. From the fact that when a nitrogen carrier was included as a part of the treatment with sulfur, its nitrification was depressed considerably as compared with that taking place when either dried blood or ammonium sulfate was included in the soil mixture without sulfur, it is shown that the natural basicity was only slightly in excess of the sulfur acidity requirement. The calcium in solution where rock phosphate and sulfur were included together is nearly equivalent to the increased phosphorus soluble in neutral ammonium citrate.

The additions of phosphorus and sulfur made to the peat soils were less than for the other soil series, 0.5 gm. of these materials being supplied. This soil is considered to be acid, and the considerably higher nitrogen figures when calcium carbonate was added support this opinion. The results for peat soil mixtures are given in table 5. It will be noted that the oxidation of

TABLE 4

*Effects of sulfur oxidation and nitrification in basic black clay, after 19 weeks incubation.*  
*(Data expressed as parts per million of dry soil mixtures)*

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acidity*	Alkalinity*
	At beginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.
None.....	130	141	245	87	153	4	0
Sulfur, 2 gm.....	119	270	1,173	2,156	16	42	0
Dried blood, 4 gm.....	130	134	668	90	491	0	3
Dried blood, 4 gm.; sulfur, 2 gm.....	129	244	1,763	2,225	12	17	0
Ammonium sulfate, 4 gm.....	125	156	1,297	1,696	280	0	7
Ammonium sulfate, 4 gm.; sulfur, 2 gm.	124	259	2,080	3,480	42	30	0
Rock phosphate, 8 gm.....	140	160	249	71	153	0	8
Rock phosphate, 8 gm.; sulfur, 2 gm....	129	479	1,746	2,184	0	52	0
Rock phosphate, 8 gm.; dried blood, 4 gm.....	142	145	702	114	516	0	3
Rock phosphate, 8 gm.; dried blood, 4 gm.; sulfur, 2 gm.....	145	323	1,849	2,398	12	23	0
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.....	153	180	1,668	2,267	326	0	2
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.; sulfur, 2 gm. ....	139	240	2,395	3,649	40	27	0

\*Acidity expressed as  $H_2SO_4$  and alkalinity as  $CaCO_3$ .

TABLE 5

*Sulfur oxidation and availability of phosphorus in acid peat soil mixtures, after 19 weeks incubation.* (Data expressed as parts per million of dry mixtures.)

ADDITIONS TO 500 GM. PEAT	CITRATE-SOLUBLE PHOSPHORUS	WATER-SOLUBLE			
		Calcium	Sulfur	Nitric nitrogen	
		p. p. m.	p. p. m.	p. p. m.	p. p. m.
None.....	356	264	118	255	
Calcium carbonate, 5 gm.....	358	737	108	556	
Sulfur, 0.5 gm.....	365	657	937	105	
Sulfur, 0.5 gm.; calcium carbonate, 5 gm.....	365	697	818	112	
Sulfur, 0.5 gm.; calcium carbonate, 2.5 gm.....	335	697	942	80	
Sulfur, 0.5 gm.; calcium carbonate, 1 gm.....	335	641	981	58	
Rock phosphate, 0.5 gm.....	339	258	108	210	
Rock phosphate, 0.5 gm.; calcium carbonate 5 gm.....	365	737	87	560	
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm. ....	368	673	952	100	
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 5 gm.....	339	818	1,025	108	
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 2.5 gm.....	366	705	935	98	
Rock phosphate, 0.5 gm.; sulfur, 0.5 gm.; calcium carbonate, 1 gm.....	389	601	881	105	

sulfur in the peat soil to which no calcium carbonate was added has increased the soluble calcium concentration. This is interpreted as resulting from the action of sulfur acidity on calcium, present in other forms than basic calcium compounds. The oxidation of sulfur in contact with rock phosphate in these peat mixtures has not increased the soluble calcium over that taken into solution from the peat mixtures which included no other treatment than sulfur. Neither do the results for phosphorus furnish indication of any action of oxidized sulfur on rock phosphate in the peat soil.

#### EFFECT OF OXIDATION OF SULFUR USED WITH NITROGEN CARRIERS

A series of soil mixtures, in which either dried blood or ammonium sulfate was included with sulfur and rock phosphate as a part of the treatment, were incubated for the purpose of studying the combined action of sulfofication and nitrification on rock phosphate in the presence of calcium carbonate as well as in the soil which contains no carbonate and possesses the characteristics of soils which are regarded as acidic on account of their deficiency in bases. The basic soil, without addition of calcium carbonate, was also used as a medium for the same treatments without the addition of calcium carbonate.

The results showing effect of the oxidation of sulfur, when used with nitrogen carriers, on rock phosphate in an acid soil are presented in table 6. The effect of similar treatment in the basic clay is shown by the data included in table 4.

When sulfur and blood were in contact in the acid soil, the oxidation of sulfur proceeded actively. Nitrification, however, in the absence of calcium carbonate, was practically inhibited by the acidity resulting from oxidation of sulfur. The transition from proteid to other forms of nitrogen proceeded to a slight extent only beyond ammonia, as the conditions were not favorable for the further change to nitric nitrogen.

The ammonia formed has apparently partially neutralized the acidity produced by the activities of the sulfofying organisms, since the results obtained for phosphorus, when dried blood was added to rock phosphate and sulfur in the soil mixtures, show that considerably less phosphorus was changed into a form soluble in neutral ammonium citrate, than where sulfur and rock phosphate were in contact without the addition of dried blood.

Where ammonium sulfate was included with rock phosphate and sulfur there was a further decrease in the amount of citrate soluble phosphorus. This effect on the solubility of rock phosphate when ammonium sulfate was used could not be due to a neutralization of acidity.

In the basic clay the effect of dried blood when used with sulfur has not been as marked as in the acid soil.



TABLE 6

*Results showing influence of the oxidation of sulfur during 19 weeks incubation, when used in combination with nitrogen carriers, on rock phosphate in acid silt loam soil. (Data expressed as parts per million of dry soil mixtures)*

ADDITIONS TO 500 GM. SOIL	CITRATE-SOLUBLE PHOSPHATE		WATER-SOLUBLE				ACIDITY*	ALKALINITY*
	At beginning	At end 19 weeks	Calcium *	Sulfur	Nitrate nitrogen	Ammoniacal nitrogen		
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Rock phosphate, 8 gm.; sulfur, 2 gm...	50	630	728	1,900	6	65	192	0
Rock phosphate, 8 gm.; dried blood, 4 gm.....	90	91	366	64	338	27	0	2
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.....	80	279	798	2,666	6	361	254	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 4 gm.....	62	62	1,295	1,628	161	225	27	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 2 gm.....	57	57	917	1,977	18	313	69	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; dried blood, 4 gm.; calcium carbonate, 1 gm.....	59	323	694	1,926	8	340	112	0
Rock phosphate, 8 gm.; ammonium sulfate, 4 gm.....	58	110	429	1,904	56	1,211		
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.....	69	179	645	2,666	8	1,486	0	3
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.; calcium carbonate, 8 gm.....	55	94	4,107	2,596	823	36	13	0
Rock phosphate, 8 gm.; sulfur, 2 gm.; ammonium sulfate, 4 gm.; calcium carbonate, 4 gm.....	64	61	2,855	2,711	365	468	0	4

\*Acidity expressed as  $H_2SO_4$ , and alkalinity as  $CaCO_3$ .

#### *Nitrification and availability of phosphorus*

The results for nitrification as affecting the availability of phosphorus which are given in table 7 show that the process proceeded more actively when the basicity of the soil was increased. Addition of calcium carbonate to this soil has stimulated the nitrification of its natural nitrogen supply, as well as that furnished by additions of dried blood and ammonium sulfate.

When calcium carbonate was not present, dried blood was nitrified to a greater extent than ammonium sulfate, the accumulation of nitric nitrogen from ammonium sulfate being less than was present in the untreated acid soil. The amount of nitrate nitrogen in the untreated soil was 84, and that produced from dried blood 294 parts per million.

The increased base requirement for the formation of nitrates from ammonium sulfate, as compared with dried blood, is clearly shown by the amount of nitric nitrogen produced from these two forms of nitrogen when the same amounts of calcium carbonate are used, that for dried blood being 462 parts per million, in contrast to only 100 parts from ammonium sulfate. Calcium carbonate was added to these mixtures at the rate of 4000 parts per million.

In soil mixtures receiving an addition of calcium carbonate at the rate of 8000 parts per million (which is double the lime requirement of the soil),

TABLE 7

*Effect of nitrification in acid silt loam soil, after 19 weeks incubation. (Data expressed as parts per million of dry soil mixtures)*

ADDITIONS TO 500 GM. DRY SOIL	CITRATE-SOLUBLE PHOSPHORUS		WATER-SOLUBLE			Acid- ity*	Alka- linity*
	At be- ginning	At end 19 weeks	Calcium	Sulfur	Nitric nitrogen		
	p. p. m.	p. p. m.	p. p. m.	p. p. m.	p. p. m.		
None .....	45	60	111	37	84	0	3
Calcium carbonate, 2 gm.....	48	54	270	50	128	0	14
Dried blood, 4 gm.....	53	63	255	58	294	0	2
Dried blood, 4 gm.; calcium carbonate, 2 gm. ....	51	54	736	95	462	0	14
Ammonium sulfate, 4 gm.....	42	81	355	1,852	54	0	0
Ammonium sulfate, 4 gm.; calcium car- bonate, 4 gm.....	42	95	2,198	1,689	569	0	3
Ammonium sulfate, 4 gm.; calcium car- bonate, 2 gm.....	45	49	1,070	2,070	100	0	6
Rock phosphate, 8 gm.....	53	98	114	42	105	0	2
Rock phosphate, 8 gm.; calcium car- bonate, 2 gm.....	67	96	280	64	128	0	14
Rock phosphate, 8 gm.; dried blood, 4 gm.....	90	91	366	64	338	0	1
Rock phosphate, 8 gm.; dried blood, 4 gm.; calcium carbonate, 2 gm. ....	63	97	667	93	394	0	13
Rock phosphate, 8 gm.; ammonium sul- fate, 4 gm.....	58	110	429	1,904	54	0	3
Rock phosphate, ammonium sulfate, 4 gm.; calcium carbonate 4 gm.....	59	95	2,058	1,919	424	0	4

\*Acidity expressed as  $H_2SO_4$ , and alkalinity as  $CaCO_3$ .

independent of that required for neutralizing acidity developed by the oxidation of either nitrogen or sulfur, the formation of nitrate nitrogen from ammonium sulfate exceeded the amount produced from dried blood.

Rock phosphate in the absence of calcium carbonate has slightly favored the nitrification of dried blood but not that of ammonium sulfate in the case of this acid soil. The amount of nitric nitrogen in the ammonium sulfate mixture is in this case the same as that produced from ammonium sulfate without rock phosphate.

Although the nitrification of dried blood was stimulated somewhat by the presence of rock phosphate and an increased amount of calcium was found in the water solution, the figures for phosphorus do not give any indication of the solvent action of nitrous acid on tricalcium phosphate. Granting that any change with respect to the phosphorus may have been masked by the adsorption capacity of the soil, and considering that the increased calcium more correctly reflects any interaction which occurred, the quantity of phosphorus as dicalcium phosphate equivalent to the increased calcium is only 86 parts per million of soil. Ammonium sulfate, from the indications furnished by the calcium as well as phosphorus, has slightly affected the solubility of tricalcium phosphate, but whatever action ammonium sulfate had is attributed to the sulfate ion rather than to biochemical action, since active nitrification of ammonia did not occur in the acid soil unless calcium carbonate was added.

The fact that more calcium from calcium carbonate was taken into solution from soil treated with ammonia sulfate than by the sulfur acidity following oxidation of sulfur, can be explained by the nitric nitrogen figures when the largest addition of calcium carbonate was necessary to furnish sufficient base for active nitrification; and by the sulfate ion where one half this quantity of calcium carbonate was not adequate for the formation of any considerable amount of nitric nitrogen from ammonium sulfate.

In the absence of rock phosphate or calcium carbonate, the nitrification of dried blood, as well as the action of ammonium sulfate without oxidation of its nitrogen, has increased the soluble calcium content. This is evidence that the natural calcium of this soil, existing chiefly as silicates, and partly in other combinations is almost, if not altogether, as readily attacked as rock phosphate. In considering the functioning of rock phosphate as a base and as promoting nitrification, it should be stated that the rock phosphate used contained a small amount of calcium carbonate, approximately 3 per cent.

The data for the basic soil mixtures which are given in table 4, show that there was active nitrification of blood, which was accompanied by a greater concentration of water soluble calcium than was found for the untreated soil. Although the natural basicity of the clay soil supported a more limited nitrification of ammonium sulfate, the amount of calcium converted into calcium sulfate through its action was almost double the calcium changed into a water soluble form through the instrumentality of the nitrifying process where dried blood was present.

Including rock phosphate as a part of the treatment has slightly increased the production of nitric nitrogen from both dried blood and ammonium sulfate, and it is assumed that the slightly larger amounts of calcium, which accompanied the increased accumulation of nitrates in the basic soil, were obtained from the rock phosphate.

In the group of mixtures where rock phosphate was added to a peat soil, no additions of dried blood or ammonium sulfate were made. The quanti-

ties of nitric nitrogen produced in the peat where rock phosphate was added, furnish no indication that it has supplied basicity necessary for the nitrifying process. Where calcium carbonate was added without sulfur, there was an increased accumulation of nitrates. The solubility of rock phosphate incorporated with the peat, does not appear to have been changed.

The results for citrate soluble phosphorus, water soluble calcium, and nitric nitrogen, do not indicate that rock phosphate incorporated with soil has been freely acted upon by the products formed during the transformation of either organic nitrogen or ammonium sulfate to nitrates. By contrasting the phosphorus availability where nitrogen carriers were included in the soil mixtures with those where sulfur was in contact with rock phosphate, it will at once be apparent how feeble the action of nitrification has been.

So far as the data which has been obtained under the experimental conditions described furnishes any information, the nitrification of dried blood and ammonium sulfate as an agency for rendering the tricalcium phosphate of floats available in amount sufficient for the requirement of plants must be regarded as a contributing factor rather than as a factor adequate in itself.

More significance should probably be attached to nitrification as an indirect agency, in conjunction with rock phosphate, for increasing crop yields. With a supply of available nitrogen furnished, plant growth is stimulated so that the phosphorus of rock phosphate can be utilized to better advantage.

Field results at the Ohio Station (5) confirm this opinion. Where nitrate of soda was added to soil which had received heavy applications of rock phosphate, the yield of wheat was much larger than that produced by rock phosphate without a supply of available nitrogen.

#### SUMMARY

The effects of sulfofication and nitrification on the availability of rock phosphate have been studied by incorporating sulfur, dried blood and ammonium sulfate with rock phosphate in soil.

In considering the influence biochemical processes may have on the availability of inert phosphates, the fact that larger additions were made than would be practical for soil application does not preclude the possibility of similar reactions occurring, although less actively, in field soils.

In an acid soil the oxidation of sulfur proceeded vigorously, approximately 50 per cent being changed to form of sulfate.

While sulfofication was somewhat depressed in an acid soil by the addition of calcium carbonate, in sand mixtures the presence of calcium carbonate was essential.

In the absence of other bases the calcium of rock phosphate did not serve as a base for the sulfofying process to any appreciable extent.

The proportion of rock phosphate to soil was such that phosphorus was added at the rate of 1900 parts per million. The oxidation of sulfur incor-

porated with rock phosphate in the absence of calcium carbonate or nitrogen carriers, has changed 630 parts of phosphorus into a form soluble in neutral ammonium citrate solution.

When calcium carbonate was added to the mixture prepared with an acid soil, the oxidation of sulfur had practically no effect on rock phosphate.

In a basic soil, the acidity resulting from sulfofication was partially neutralized by calcium naturally present as carbonate and in other combinations, so that the solvent action on rock phosphate was much less than occurred in the acid soil.

Ammonium sulfate affected the solubility of rock phosphate very little. Whatever action ammonium sulfate has had, is attributed to the sulfate ion, rather than to biochemical action, since nitrification of ammonia did not occur in a soil deficient in bases unless calcium carbonate was added.

Active nitrification of dried blood and ammonium sulfate occurred in the mixtures when conditions were favorable.

Nitrification has been stimulated by rock phosphate to a very limited extent. This fact, independent of the results for either phosphorus or calcium solubility, is sufficient indication that the process has had no appreciable action on rock phosphate in soil.

Nitrification of dried blood, so far as the citrate soluble figures furnish evidence of availability, is not an active agent for increasing the solubility of rock phosphate mixed with soil.

In the absence of rock phosphate or calcium carbonate, the nitrification of dried blood as well as the action of ammonium sulfate, independent of the oxidation of its nitrogen, has increased the concentration of water soluble calcium. More calcium has been taken into solution from the soil than from added rock phosphate. This is evidence that the calcium of the soil, existing chiefly as silicates and partly in other combinations is almost, if not altogether, as readily attacked as rock phosphate.

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# ORGANIC PHOSPHORUS OF SOIL: EXPERIMENTAL WORK ON METHODS FOR EXTRACTION AND DETERMINATION

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## INTRODUCTORY

In pursuance of investigations pertaining to the availability and combinations of the phosphorus in Ohio soils, begun in 1916, the need for a method, by means of which the inorganic and the organic phosphorus of soil extracts may be differentiated, became apparent. A limited amount of experimental work had already been devoted to modification of the methods of Forbes and associates (4) for inorganic phosphorus in plant substances, with some encouraging results, when Potter and Benton's (15) adaptation of the Forbes and Emmet and Grindley (3) method for inorganic phosphorus determinations appeared.

The procedure of Potter and Benton has been made the subject of a critical study, having in view the determination of the conditions necessary for best results and the simplification of the method, if possible.

Some attention has also been devoted to the determination of total phosphorus in alkali extracts of soil; obviously, in any study of the organic phosphorus present in such extracts, where the latter figure must be obtained by difference, the accuracy of each determination is equally important.

An effort has been made to establish the conditions under which the maximum amount of organic phosphorus can be extracted from the soil, and to prove that the extracted phosphorus as determined to be organic is really in that state of combination and not inorganic phosphorus absorbed or held in an insoluble state by suspended matter, organic or inorganic.

The total organic matter (humus) content, comparative intensity of color and principal ash constituents have been determined on a number of the ammonia extracts obtained during the course of this study in the hope that a clue to their relationship to the phosphorus content might be obtained.

## EXPERIMENTAL

### *Soil*

The soil employed for this investigation was from a large lot taken for vegetation tests from the Paulding County (Ohio) experiment farm. It was formerly classed as a Clyde clay, now called "Fulton clay" by the Ohio Soil

Survey. The following quotation is from Bulletin 323 of the Ohio Station, to which the interested reader is referred for information relative to crop yields and fertility tests on this soil:

This soil is a dark-colored, fine-textured material, water leveled over a tough glacial till, which usually forms the lower subsoil and is therefore nearly level. It has the greatest amount of available plant-food of all soils in the state.

A partial analysis of a sample of this soil, taken in close proximity to the place where the larger sample used in this investigation was obtained, is presented in table 1.

As may be seen by a comparison of the figures for ammonia-soluble organic matter and phosphorus with those presented in later tables, the sample analyzed differs somewhat in composition from the lot used in the work to be

TABLE 1  
*Composition of soil from Paulding County Experiment Farm*

DEPTH	PHOSPHORUS			POTASSIUM		CALCIUM		MAGNESIUM		CaCO <sub>3</sub>	NITROGEN	NH <sub>4</sub> OH — SOLUBLE HUMUS
	Total	N/5 HNO <sub>3</sub> — sol- uble	NH <sub>4</sub> OH — sol- uble organic	Total	N/5 HNO <sub>3</sub> — sol- uble	Total	N/5 HNO <sub>3</sub> — sol- uble	Total	N/5 HNO <sub>3</sub> — sol- uble			
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent			
<i>inches</i>										<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0-6	0.098	0.021	0.034	2.336	0.025	0.943	0.525	0.717	0.045	0.023	0.350	3.294
6-12	0.078	0.017	0.026	2.555	0.021	0.849	0.457	0.776	0.031	0.029	0.232	2.066
12-18	0.066	0.018	0.019	2.604	0.017	0.797	0.437	0.789	0.066	0.058	0.177	1.460
18-24	0.062	0.018	0.012	2.520	0.017	0.745	0.385	0.801	0.072	0.055	0.130	1.034

described, but this should be of no consequence for present purposes, since the larger lot was all taken from the same place and had been thoroughly mixed.

This particular soil was selected for the present investigation on account of its high content of organic matter and phosphorus, and the fact that a considerable supply of it was available.

### *Methods of analysis*

*Inorganic phosphorus.* As the result of considerable experience with the method for inorganic phosphorus in humus solutions substantially as described by Potter and Benton, except that concentration of the neutralized nitric acid extract of the first magnesia mixture precipitate was omitted as unnecessary (it being easily possible with care to secure complete extraction and still not greatly exceed the volume of 125 cc. specified by the authors named), as well as the same method variously modified otherwise, the writer has adopted the following procedure:

Pipette a suitable aliquot (200 cc., representing 20 gm. of soil) of the humus extract into a 500-cc. centrifuge bottle, add 25 cc. of standard phosphate solution (approximately 0.0025 gm. P) and precipitate with 25 cc. of magnesia mixture, added drop by drop with stirring. Shake well and add strong ammonia to bring the concentration of  $\text{NH}_3$  in the final volume of solution up to 2.5 per cent. Stopper and let stand three days. Whirl in the centrifuge at 3000 revolutions per minute for 10 or 15 minutes. Pour the supernatant liquid through an 11-cm. filter (Swedish Paper No. 0-B is satisfactory) carefully fitted into a 60° long-stemmed funnel with platinum cone in bell jar, and wash the inside of the bottle and surface of the precipitate once with cold water, being careful not to disturb the cake of precipitate. After pouring this through the paper, the latter may be washed several times with water and a little filter paper pulp added to the filter. Add to the precipitate in the bottle 25 or 30 cc. of dilute nitric acid (80 cc. of acid of specific gravity 1.2 diluted to 1000 cc.), shake well and allow to stand for a few minutes. Pour into the filter and receive the filtrate in a 250-cc. beaker covered with a perforated watch-glass under a bell jar; if the filtrate is not perfectly clear, it should be returned to the filter. Wash the bottle and precipitate with five or six more portions of the dilute nitric acid, using 15 or 20 cc. each time.

The filtration should not be unduly hastened at this time and it is better to use only enough suction to keep the stem of the funnel filled. The chances of loss will then be less, while the acid has an opportunity to diffuse into the precipitate and extract the soluble phosphorus with a minimum amount of washing. For the same reason, the funnel should be allowed to empty each time, but should then be refilled at once, otherwise the precipitate may become compact and crack, allowing channels to form.

The filtrate is neutralized with strong ammonia, and 8 cc. of nitric acid, specific gravity 1.2, is added; the volume should not exceed 175 cc. Add about 10 gm. of solid ammonium nitrate, heat to 60°C., add 50 cc. of warm neutral molybdate (75 gm. of crystallized ammonium molybdate per liter, dissolved with the aid of a little ammonia and neutralized with  $\text{HNO}_3$ ) or 50 cc. of ordinary acid molybdate neutralized to litmus paper with ammonia, and maintain at 60° for about one-half hour. In case the latter is used, it is not desirable to add any solid ammonium nitrate. Remove from the bath and let stand several hours or over night. Filter, wash well with cold 1 per cent  $\text{NH}_4\text{NO}_3$  slightly acidified with nitric acid, and dissolve back into the same beaker with 2.5 per cent  $\text{NH}_3$ , followed by washing with water, but keeping the volume as small as possible. Neutralize with  $\text{HCl}$ , add 5 cc. of 5 per cent citric acid solution to aid in keeping in solution any iron which may be present, make slightly alkaline with ammonia and precipitate according to the usual procedure with 10 cc. of magnesia mixture and one-tenth volume (about 10 cc.) of strong ammonia. After standing over night, the precipitated magnesium ammonium phosphate is filtered off, using a close-grained paper, as the precipitate may be in a very finely-divided condition, thoroughly washed with 2.5 per cent ammonia, redissolved with dilute hydrochloric acid into a clean beaker, keeping the volume of the solution below 100 cc. if possible. The phosphorus is reprecipitated by magnesia mixture as before, and after standing over night the white precipitate is filtered off on dense ashless paper (blue ribbon quality), thoroughly washed with 2.5 per cent  $\text{NH}_3$  (one volume of strong ammonia, diluted to 10 volumes is sufficiently near the exact strength) and finally ignited and weighed as  $\text{Mg}_2\text{P}_2\text{O}_7$ . The weight of phosphorus is calculated from this, that corresponding to the phosphate solution added and the blank being deducted to obtain the net content of inorganic phosphorus of the 200-cc. aliquot taken. If desired, the determination might just as well be finished by any of the standard methods of weighing or titrating the yellow precipitate, beginning with the washed white precipitate following the neutral molybdate separation.

The writer's experience has been that it is not necessary to make any special effort to remove silica; the repeated precipitations and washings accomplish this perfectly, and none has ever been found in the final precipitate.



The above-described method differs very materially in several respects from that published by Potter and Benton; no ammonium chloride except that contained in the magnesia mixture and but one-half the quantity of the latter reagent prescribed by the authors named, is required. The object of this substitution is to avoid as far as possible, the "salting out" of the humus bodies and the consequent increase in difficulty of working with the larger precipitate. Reducing the proportion of magnesia mixture still further would reduce the amount of organic matter precipitated to an almost negligible quantity, but unfortunately cannot be done, as recovery of the inorganic phosphorus is then incomplete. The strength of ammonia employed and the time allowed are both much greater than advised in the original method; as will be shown subsequently, practically the same strength of ammonia and almost as much time are required to secure maximum extraction of the organic phosphorus compounds of the soil under investigation. In so far as stability is concerned, there has been no sensible increase in the content of inorganic phosphorus in a number of 4 per cent ammonium hydroxide extracts after standing several months.

The single washing of the first precipitate as directed in the procedure outlined is easily accomplished and seems to be all that is necessary; if thorough washing with water or dilute ammonia not containing a considerable amount of magnesia mixture is attempted, the precipitate begins to dissolve after the greater part of the salts present is removed, and it is possible with patience to wash practically all the organic matter out. The precipitated organic matter does not seem to have formed any definite compound with any of the reagents, but has merely been coagulated by the considerable concentration of salts.

The addition of a known amount of phosphate is for the purpose of having sufficient inorganic phosphorus present to enable that already present to precipitate. This would be unnecessary in case a soil giving an ammonia extract containing a considerable amount of inorganic phosphorus was being worked with.

In illustration of several of the points which have been mentioned, the following account of the distribution of the phosphorus in the several stages of the determination of inorganic phosphorus will be of interest.

In the determination 200-cc. aliquots of a humus solution, containing in that quantity 9.8 mgm. of total phosphorus, were treated as described, phosphorus being added to each, but 25 cc. and 10 cc., respectively, of magnesia mixture used; all were made to 2.5 per cent  $\text{NH}_3$  and stood three days before centrifuging and filtering.

In table 2 account should be taken of the added phosphorus, which has not been deducted from figures representing the inorganic phosphorus found.

The results for inorganic phosphorus from the determinations in which 25 cc. of magnesia mixture were employed, besides being of a very different order of magnitude from those obtained by the use of but 10 cc. of the re-

agent, show a better agreement. In fact, the lack of agreement between the duplicates and the fact that in the case of two determinations in which the minimum quantity of magnesia mixture was employed, the amounts of inorganic phosphorus found are less than the amounts added, is evidence that the results of these determinations are of a lower degree of accuracy, although not absolute proof that the results are incorrect, since a poor method will often afford closely-agreeing results and in any method involving so many successive reprecipitations of a small quantity of the element determined, it may be expected that the tendency will be toward low results.

The figures obtained from determinations of total phosphorus in the filtrates from the first precipitation by magnesia mixture indicate that the larger amount of the reagent has precipitated about 60 per cent of the organic phosphorus along with the inorganic, while in the case of those determinations

TABLE 2  
*Distribution of phosphorus in the determination of inorganic phosphorus*

MAGNESIA MIXTURE USED	INORGANIC PHOSPHORUS		"ORGANIC PHOSPHORUS"		
	Added	Found	Not precipitated	Remained in precipitate	Leached out
cc.	mgm.	mgm.	mgm.	mgm.	mgm.
25	2.5	3.9	3.7	4.5	0.2
	2.5	3.9	3.6	4.8	
	5.0	6.4			
	5.0	6.5			
10	2.5	2.7	8.8	0.6	0.2
	2.5	2.3	9.3	0.6	0.1
	5.0	4.4			

in which the precipitation was made by 10 cc. of magnesia mixture, only about 8 per cent of the apparent content of organic phosphorus was precipitated.

J. Stewart (21) appears to have been the first to apply the magnesia mixture precipitation procedure of Forbes and associates to the determination of inorganic phosphorus in alkali extracts of soil; he was led to abandon the method on account of the considerable solubility of the organic matter precipitated by magnesia mixture in the acid-alcohol prescribed by the Forbes method, and the large proportion of the total phosphorus precipitated by magnesia mixture. The present writer's experience with the acid-alcohol extraction of the Forbes method has been exactly that of Stewart; not only is the extract highly colored, but a voluminous precipitate of organic matter, iron, etc. appears when a second precipitation by magnesia mixture is attempted, while a gummy residue is left on evaporation. The second objection to the magnesia precipitation method would have more weight if it were shown that any considerable part of the organic phosphorus in the magnesia mixture

precipitate is extracted by the dilute acid employed for leaching out the inorganic phosphorus and carried on to the next stage; fortunately, the dilute nitric acid substituted by Potter and Benton for the acid alcohol of Forbes is not open to this objection, as shown by the data in table 2. Of the phosphorus precipitated by the larger amount of magnesia mixture, the greater part remains with the insoluble organic matter after leaching with acid, as shown by total phosphorus determinations following wet digestions of the leached precipitates. The figures for organic phosphorus taken into solution by the leaching with dilute acid were obtained by difference; apparently no more organic phosphorus was dissolved during the acid leaching when much was present in the precipitate leached than when but little was present.

A possibility that the larger amount of inorganic phosphorus found when 25 cc. of magnesia mixture was used is due to organic phosphorus taken into solution during the acid leaching and decomposed during subsequent operations, must be considered. As evidence again this, the following observations are presented.

1. In working with similar solutions of about the same organic phosphorus content, but containing less inorganic phosphorus and to which none had been added in the course of the determination of inorganic phosphorus, as large a precipitate of organic matter resulted as was encountered when much inorganic phosphorus was present. Nevertheless, even when magnesia mixture was used at the rate of 40 cc. per 200 cc. of humus solution, the determination of inorganic phosphorus showed a mere trace or none at all. As the next step in the analysis, the neutral molybdate precipitation, has always been found capable of showing the presence of a very minute amount of inorganic phosphorus, it is evident that the decomposition of organic phosphorus compounds has been a negligible factor in these cases.

2. The ammonia extract, with which the results given in table 2 were obtained, was a mixture of extracts left from other work, in which known amounts of inorganic phosphorus were added to suspensions of acid-extracted soil in ammonia previous to filtration. While there is no means of knowing the proportion of inorganic phosphorus present in this mixture, except as the result of the analyses which have been discussed, all the data at hand indicate that it was greater in amount than the maximum shown when only 10 cc. of magnesia mixture was depended upon to precipitate it.

For a time, the writer made a practice of using the minimum quantity of magnesia mixture for this work, both for the reasons discussed by Stewart and on account of the ease with which the filtration and leaching by dilute acid are accomplished when the precipitated inorganic phosphorus is accompanied by little organic matter; the impossibility of obtaining consistent results, no matter how well the duplicate determinations agreed, was proof that something in the procedure was at fault.

The very small amount of organic phosphorus which accompanied the inorganic phosphorus in the nitric acid leachings of the first magnesia mixture

precipitate cannot have much influence upon the final results, even if all of it should appear as inorganic phosphorus. The ordinary acid molybdate separation is therefore found to be satisfactory in work with this soil. Fair results were also obtained when the molybdate separation was entirely omitted, a second precipitation by magnesia mixture in presence of citric acid to hold iron in solution being substituted.

This second precipitate of magnesium ammonium phosphate is but slightly contaminated by organic matter if care has been taken to insure a clear acid extract of the first, and was ignited and weighed directly.

If subsequent work should show that phytin or similar phosphorus compounds occur in alkali extracts of soil, the acid-alcohol separation could doubtless be successfully applied to this second precipitate.

*Total phosphorus.* Both the magnesium nitrate method of ignition and the wet combustion (Neumann) method have been used in obtaining the data for total phosphorus in alkali extracts of soil reported in this paper. The latter method is preferred, and was employed in the great majority of cases. It offers the advantages of total elimination of silica, no liability to loss by deflagration and, if properly conducted, none from other causes. The procedure followed is described:

Pipette a 200-cc. aliquot of the humus solution into a 500-cc. long-necked Kjeldahl flask, acidify with concentrated hydrochloric acid, add 50 cc. of concentrated nitric acid and 5 cc. of concentrated sulfuric acid. Drop in several bits of broken glass or beads as a preventive of bumping and place in the neck of the flask an "arrester" made by blowing a bulb of a size to fit loosely in the neck of the flask on the end of a piece of glass tubing and cutting the latter about six inches from the bulb; the tubing is bent at right angles about two inches from the end and one then has an arrangement which may be placed in the neck of the flask and held there by the short arm bent at a right angle when placed on the digestion rack with the mouth of the flask in the opening of the fume duct. The object is to prevent loss by spurling, to which these digestions are very liable, on account of the solid matter which separates during the later stages of the digestion.

The flask may be heated with a full flame from the beginning and the boiling proceeds without troublesome foaming or bumping. The solution should be allowed to boil until the sulfuric acid becomes concentrated, when the organic matter will be charred and copious fumes evolved. Turn out the flame and allow to cool for about 5 minutes; add a little concentrated nitric acid and heat as before, until the dense fumes have disappeared and only the colorless vapor of sulfuric acid is above the boiling acid. After several repetitions of this, always allowing the flask to cool somewhat before adding more nitric acid and never more than 2 or 3 cc. of this at a time, the contents of the flask should be practically colorless or only slightly tinged with yellow; allow to become quite cold, carefully dilute to 50 cc. with cold water, heat and boil gently for several minutes. Filter and wash into a 250-cc. beaker to a volume of about 125 cc. Neutralize with strong ammonia, bring any precipitate into solution again with nitric acid, add 15 gm. of solid ammonium nitrate, heat to 65°C. and precipitate with 50 cc. or more of official molybdate solution. Maintain at 65°C. for one-half hour, and allow to stand at least 3 hours longer before filtering. Filter, wash well with cold 1 per cent ammonium nitrate made slightly acid with nitric acid, dissolve from the filter with the aid of 2.5 per cent  $\text{NH}_3$ , add 5 cc. of 5 per cent citric acid solution and proceed in the customary manner for determination of phosphorus as magnesium pyrophosphate.

The small amount of sulfate present does not interfere with the complete precipitation of the phosphorus by the molybdate, provided the directions given are followed; the general effect of sulfates upon the molybdate precipitation is to cause the yellow precipitate to come down more slowly and to have an abnormal composition, necessitating more molybdate reagent and a higher temperature and longer time than would be required for the complete precipitation of phosphorus in the absence of sulfates. For this reason, the use of any of those methods for total phosphorus which depend upon the weighing or titration of the yellow precipitate itself, is not recommended unless the sulfates are first removed.

#### EXTRACTION OF ORGANIC PHOSPHORUS

In the search for a method for obtaining an index to the soil's total content of organically combined phosphorus, the use of alkalies as solvents for such phosphorus compounds suggests itself, since it is known that the alkali extracts of soils are often high in this element. In the past, much attention has been devoted to the phosphorus content of ammonia extracts of soil, and while no investigator has denied that at least a part of the phosphorus in such extracts of normal soils might be in organic combination, many explanations of its nature have been offered.

One of the most difficult features of the study of the ammonia-soluble constituents of the soil is getting rid of the clay, which remains in suspension in these extracts almost indefinitely; although it is readily enough precipitated by a sufficient addition of any one of a number of salts, there is no assurance that in precipitating it has not carried with it material from solution. On the other hand, this colloidal clay certainly contains in greater or less amount every constituent of the soil, and must be removed.

Gortner and Shaw (10) have recently laid considerable emphasis upon the relation of the clay content of alkali extracts of soil to the apparent occurrence of organic phosphorus as determined by Potter and Benton's method.

As an indication of the amount of study devoted to methods for the removal of this clay from solutions intended for humus determinations, it will suffice to say that a very considerable part of the voluminous literature upon humus has been devoted to this phase of the subject.

The following procedures for the removal of clay from ammonia extracts of soil have been studied in the present investigation:

1. Filtration through a layer of the soil itself supported by paper on a 25-cm. Büchner funnel, without any precipitant for the clay.
2. Centrifuging at 3000 revolutions per minute for 10 minutes in International Instrument Company's no. 3 laboratory centrifuge.
3. One passage through Sharples laboratory centrifuge running at 30,000 revolutions per minute.
4. Powdered ammonium carbonate added at rate of 2 gm. per liter, centrifuged like no. 2.

5. The same, 10 gm. per liter.
  6. The same, 2 gm. per liter, filtered on a 25-cm. Büchner funnel.
  7. The same, 10 gm. per liter.
  8. Ammonium chloride added at the rate of 10 gm. per liter, centrifuged like no. 2.
  9. Hydrogen sulfide passed into the suspension of soil in ammonia until clay was precipitated, centrifuged like no. 2.
  10. The same, filtered on a 25-cm. Büchner funnel.
- These humus extracts were prepared as follows:

One kilogram of soil was placed in a large bottle and digested with frequent shaking for several hours with 2 per cent HCl. It was then thrown upon a 25-cm. Büchner filter and washed with 1 per cent HCl until no calcium could be detected in a 50-cc. portion of the leachings. The soil was washed with water until the washings were entirely free from chlorine, sucked as dry as possible, and the cake of soil removed from the funnel and allowed to become air-dry, then reground. Then 85-gm. portions of this prepared soil were weighed into liter bottles, 850 cc. of 4 per cent  $\text{NH}_4\text{OH}$  added, and the mixture shaken during 4 working days in an end-over-end shaker revolving about 10 times per minute. The clay was thereafter separated as quickly as possible.

In table 3, the determinations made on these solutions are presented. The figures in the columns headed "grams" refer to the number of grams of the particular constituent per 200 cc. of humus solution. Humus and humus ash were determined by evaporating 200 cc. in a weighed platinum dish, drying at  $110^\circ\text{C}$ . for about  $1\frac{1}{2}$  days, which was found to be the length of time required to approximate constant weight, weighing, igniting and again weighing.

Silica was determined by the standard method after a carbonate fusion of the ash. Alumina was determined by a modification of the Peters method, as described by Blair (2), and ferric oxide was determined by the colorimetric method described by Schreiner and Failyer (18) in a small aliquot of the filtrate from the silica. The humus solutions were compared as to color by diluting a suitable aliquot to 100 cc. and making the comparisons in a Schreiner colorimeter. Total and inorganic phosphorus were determined by the methods previously described.

The data for humus, presented in table 3, indicate that in those cases where centrifuging has effected a good removal of clay, the salts added to bring this about have caused a decrease in the content of organic matter in solution. The same effect is apparent in the figures for comparative color, and to an even greater extent in those for total phosphorus.

Centrifuging without the addition of any precipitant for clay, or only the minimum quantity of ammonium carbonate, gives high results for all constituents; in the case of humus, this is certainly partly due to combined water in the mineral matter. Considering the large amount of clay left in solution, the increase in the phosphorus content over a solution filtered without any precipitant is not large. Filtration after the addition of precipitants causes decreases in total organic matter, color and total phosphorus; the only ex-

ception to this is the solution treated with the minimum amount of ammonium carbonate, which has shown an increase in total organic matter. This effect of ammonium carbonate has been studied by MacIntire and Hardy (14), and is attributed by them to a solvent action upon a part of the organic matter of the soil which is not entirely dissolved by ammonia alone. With the larger amount of ammonium carbonate, this effect is masked by the tendency of high concentrations of the precipitant to cause occlusion of organic matter by the precipitated clay.

TABLE 3

*Constituents of 200 cc. of 4 per cent  $\text{NH}_4\text{OH}$  extract, representing 20 gm. of acid-extracted soil*

TREATMENT	HUMUS	COLOR	ASH	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$\text{Fe}_2\text{O}_3$	RATIO OF $\text{SiO}_2$ TO $\text{Al}_2\text{O}_3$	TOTAL PHOS- PHORUS
	gm.		gm.	gm.	gm.	gm.		gm.
1. Filtered	0.7800	100	0.0632	0.0113	0.0025	0.0156	4.5	0.0090
2. Centrifuged	0.9070	125 <sup>+</sup>	0.5718	0.2874	0.1350	0.0549	2.1	0.0095
3. Centrifuged	0.9185	109 <sup>+</sup>	0.8599	0.4330	0.2055	0.0432	2.1	0.0092
4. 0.2 per cent $(\text{NH}_4)_2\text{CO}_3$ centrifuged	0.8686	114 <sup>+</sup>	0.4382	0.2034	0.0960	0.0340	2.1	0.0093
5. 1.0 per cent $(\text{NH}_4)_2\text{CO}_3$ centrifuged	0.7755	95	0.0765	0.0215	0.0061	0.0119	3.5	0.0077
6. 0.2 per cent $(\text{NH}_4)_2\text{CO}_3$ filtered	0.7942	100	0.0647	0.0110	0.0027	0.0156	4.1	0.0087
7. 1.0 per cent $(\text{NH}_4)_2\text{CO}_3$ filtered	0.7488	89	0.0501	0.0098	0.0011	0.0109	8.9	0.0075
8. 1.0 per cent $\text{NH}_4\text{Cl}$ cen- trifuged	0.7488§	91	0.0755	0.0231	0.0071	0.0119	3.3	0.0075
9. $\text{H}_2\text{S}$ centrifuged	*	91	0.0903	0.0440	0.0031	0.0017	14.2	0.0080
10. $\text{H}_2\text{S}$ filtered	*	94	0.0813	0.0369	0.0028	0.0015	13.2	0.0082
11. Original solution	0.8185	104	0.0777	0.0171	0.0032	0.0280	5.3	0.0092
12. Same filtered through bou- gie of porous porcelain	0.7311	85	0.0683	0.0137	0.0031	0.0176	4.4	0.0086
13. First liter to pass through Büchner filter	0.8281	100	0.0722	0.0175	0.0010	0.0233	17.5	0.0095
14. Second liter collected	0.8720	100	0.0777	0.0204	0.0010	0.0233	20.4	0.0096

<sup>+</sup> Turbid from clay.

\* Sulfur compounds present in large amount.

§ Ammonium chloride added has been deducted from weight.

The alumina content of these solutions is in all probability the best index to the actual amount of clay contained in them, although it is not at all impossible that a part of the alumina may be in solution in the ammonia instead of being merely in suspension as clay. The silica is in excess, indicating that in the greater number of cases it is not merely present as clay; in solutions 2, 3, 4, the clay content of which is highest, the ratio of silica to alumina is in each case 2.1, but in all other cases the ratio is much higher. The ferric oxide content of the solutions does not appear to bear any relation to other

constituents; it is highest where most clay is present, except in the case of solution 3, which is thought to have been diluted somewhat during the centrifuging. The results on this solution are anomalous in several respects and would not be included were it not that the composition of the ash is of interest, and to show the difficulty of removing all clay even by the application of great centrifugal force.

The reduction in the iron content of these solutions by precipitation with hydrogen sulfide is striking; it was noted that solution 10 was filtered through the layer of soil and paper on the Büchner funnel with unusual rapidity, which would seem to indicate that the iron content of these ammonia extracts contributes not a little to the difficulty of working with them.

Determinations of inorganic phosphorus in these solutions gave negative results; the work was done before the necessity of adding a known amount of phosphorus was appreciated. However, it is probable that the content of inorganic phosphorus is in every case quite small, and there is no reason to suppose that it differs to any extent among the solutions in the series. The content of organic phosphorus probably differs with the amounts of total phosphorus present, the phosphorus contained in suspended clay being included with the organic phosphorus.

The conclusion drawn from the data in table 3 is, that the procedure best adapted for obtaining an ammonia extract of the soil which contains the minimum amount of clay and the maximum amount of phosphorus includes as an essential feature separation of the clay by filtration through a layer of the soil itself supported by a flat paper filter on a Büchner funnel, substantially as described by MacIntire and Hardy.

The use of salts as precipitants for the clay before filtration causes decreases in the content of phosphorus without effecting any significant reduction of the clay content from that obtained by filtration without any precipitant. The use of the minimum quantity of ammonium carbonate (2 gm. per liter) has a slight influence upon both the organic matter and phosphorus content of the filtrate and in opposite directions. While the decrease in the amount of phosphorus present is but little greater than the probable difference in duplicate determinations, other solutions prepared similarly and compared show about the same difference; it may, therefore, be accepted as a fact that even this small amount of precipitant causes a noticeable decrease in the phosphorus content of the filtrate. Filtration is greatly facilitated when ammonium carbonate is used; 2 gm. per liter has almost as much influence in this respect as 10 gm. per liter.

A method of filtration which appeared promising and which it was hoped would remove every trace of clay depended upon the use of the Pasteur-Chamberland bougie of unglazed porcelain. This was not tried directly upon the soil suspension, but upon a solution already filtered on a Büchner funnel. The comparative analyses of this solution before and after filtration through the bougie are presented in table 3, nos. 11 and 12. The soil used was from a



lot prepared at a different time, so that the results are not strictly comparable with those for the other solutions tabulated.

Filtration through porous porcelain has reduced the amounts of all constituents present in the solution; the first portions to run through were apparently unchanged, but filtration gradually became slower and the filtrate lighter in color. About a liter of the solution was thus filtered; the last 50-cc. unfiltered portion remaining in the mantle was noticeably thicker and darker in color than the original solution had been.

On account of the slowness with which the filtrate passes through the layer of soil on the Büchner funnel when no precipitant is employed, indicating that a filter of this kind has but poor permeability, it was thought possible that there might be a similar decrease of concentration in the later portions to pass the filter. Nos. 13 and 14 in table 3 are, respectively, the first and second liters collected; it will be seen that the second liter is slightly stronger, instead of weaker than the first. A similar result, for humus and humus ash only, was reported by MacIntire and Hardy. These solutions were made from still another lot of extracted soil, which will explain the difference in composition observed when they are compared with solutions previously discussed and presented in table 3.

#### *Optimum strength of ammonia*

In table 4 data obtained from experimental work to establish the most favorable strength of ammonia are presented. The soil used was from a lot of 3 kgm. prepared at one time, in the manner previously described; it will hereafter be referred to as "lot 2."

The proportion of air-dry soil to ammonia solution was 1 gm. to 10 cc., as in the work previously discussed, and the shaking in the mechanical shaker continued during four working days. The solutions were filtered without precipitant on flat papers in 25-cm. Büchner funnels, these conditions having been tentatively adopted as standard.

The concentrations of ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) employed were, respectively, 0.5, 1, 2, 4, 6, and 8 per cent in numbers 1 to 6; there is a steady increase in humus and color extracted, with increases in the strength of ammonia; total phosphorus reaches a maximum at the 6 per cent strength; and the other ash constituents show a tendency to decrease with increases in the strength of ammonia.

Inorganic phosphorus determinations were made on all these solutions; both the absolute amounts and the differences observed were too small to have much significance, hence, the results are not tabulated. The results obtained did not indicate that the higher strengths of ammonia had caused any more decomposition of organic phosphorus than the lower strengths.

The following conclusions are drawn:

Six per cent  $\text{NH}_4\text{OH}$  is but slightly, if any, more efficient than 4 per cent as a solvent for the organic phosphorus compounds in this soil; 6 per cent  $\text{NH}_4\text{OH}$  is as efficient as 8 per cent.

TABLE 4

*Constituents of 200 cc. of ammonia extract, representing 20 gm. of acid-extracted soil*

STRENGTH OF $\text{NH}_4\text{OH}$	HUMUS	COLOR	ASH	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$\text{Fe}_2\text{O}_3$	RATIO OF $\text{SiO}_2$ TO $\text{Al}_2\text{O}_3$	TOTAL PHOSPHORUS
<i>per cent</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>
0.5	0.6492	92	0.0928	0.0179	0.0057	0.0316	3.1	0.0078
1.0	0.6908	89	0.0788	0.0123	0.0010	0.0239	12.3	0.0080
2.0	0.7412	92	0.0760	0.0120	0.0018	0.0226	6.7	0.0083
4.0	0.8496	100	0.0664	0.0105	0.0012	0.0198	8.8	0.0089
6.0	0.8744	104	0.0680	0.0130	0.0014	0.0198	9.3	0.0090
8.0	0.8988	109	0.0648	0.0120	0.0015	0.0198	8.0	0.0090

*Time soil should be shaken with ammonia*

Data given in table 5 were designed to show the length of time necessary for maximum extraction of organic phosphorus.

Prepared soil from "lot 2" was employed, the standard conditions were followed, and the ammonia was 4 per cent ammonium hydroxide. The only variable factor was the length of time the soil and ammonia were shaken together in the end-over-end shaking machine.

The periods of shaking were 2, 4, 6, and 8 hours, and 2 and 4 days, respectively, for solutions 1 to 6.

Humus and color increase with the length of time shaken, the ash constituents tend to decrease, and the total phosphorus does not exhibit any significant variations.

The periods of time required for filtration varied with the time the soil and ammonia were shaken together; it is evident that the maximum deflocculation produced by 4 days' shaking has caused the layer of soil to form a

TABLE 5

*Constituents of 200 cc. of 4 per cent  $\text{NH}_4\text{OH}$  extract, representing 20 gm. of acid-extracted soil*

LENGTH OF TIME SHAKEN	HUMUS	COLOR	ASH	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$\text{Fe}_2\text{O}_3$	RATIO OF $\text{SiO}_2$ TO $\text{Al}_2\text{O}_3$	TOTAL PHOSPHORUS
<i>hours</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>
2	0.6972	88	0.0811	0.0195	0.0053	0.0368	3.7	0.0087
4	0.6957	90	0.0832	0.0223	0.0060	0.0257	3.7	0.0086
6	0.7074	91	0.0820	0.0224	0.0049	0.0263	4.6	0.0086
8	0.7715	94	0.0772	0.0208	0.0041	0.0235	5.1	0.0087
16	0.7945	98	0.0697	0.0153	0.0030	0.0227	5.1	0.0088
32	0.8173	100	0.0630	0.0133	0.0013	0.0209	10.2	0.0088

very impermeable filtering medium in the Büchner funnel, requiring a longer time for filtration and resulting in a more complete removal of clay.

In this connection, it should be noted that from one to three days' time was required for the filtrations, so that the soil and ammonia were actually in contact longer than stated. It may be, therefore, that 2 hours' shaking would not show such complete extraction of phosphorus if some quick method of separating clay had been employed.

*Optimum ratio of soil to solvent*

In order to determine what influence changes in the ratio of soil to solvent might have upon the extraction of the organic phosphorus, portions of prepared soil from "lot 2" were weighed out, representing 400, 100 and 20 gm. of moisture-free soil, and treated with ammonia solution of such volume and strength that there would be exactly 1 liter of 4 per cent  $\text{NH}_4\text{OH}$  in contact with the soil after allowing for the slight percentage of moisture in the soil and the reduction in strength of the ammonia by absorption of alkali by the acid-extracted soil. The mixtures were shaken for 8 hours and filtered in the customary manner, the contents of several bottles of the most dilute extract being filtered upon the same Büchner funnel.

TABLE 6

*Constituents of 4 per cent  $\text{NH}_4\text{OH}$  extract representing 20 gm. of moisture-free acid-extracted soil*

WEIGHT OF SOIL EXTRACTED BY ONE LITER	HUMUS	COLOR	ASH	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$\text{Fe}_2\text{O}_3$	RATIO OF $\text{SiO}_2$ TO $\text{Al}_2\text{O}_3$	TOTAL PHOSPHORUS
gm.	gm.		gm.	gm.	gm.	gm.		gm.
400	0.8309	433.0	0.0636	0.0093	0.0009	0.0238	10.3	0.0089
20	0.8734	17.5	0.1187	0.0456	0.0056	0.0268	8.1	0.0091
100	0.8185	100.0	0.0777	0.0171	0.0032	0.0280	5.3	0.0091

In table 6, data obtained from analyses of these solutions are presented; the figures in the columns headed "grams" refer to the number of grams of the particular constituent contained in a volume of solution representing 20 gm. of moisture-free soil. The figures for color represent a direct comparison of color of the extracts.

The 20-gm. per liter extract has removed most humus from the soil, but curiously enough, the 400-gm. per liter extract stands next. The latter has also extracted proportionately more color, and the 20 to 1000 solution has extracted proportionately least color. The ash, silica, alumina, and ferric oxide content of the 400 to 1000 solution is lowest per unit of soil; except for ferric oxide, the same constituents are highest in the case of the 20 to 1000 extract.

The extraction of phosphorus is a trifle less efficient when the volume of extract per unit quantity of soil is least; it has been the same, however, whether 20 or 100 gm. of soil were extracted by a liter of the solvent.

*Preliminary extraction of bases*

It is a well known fact that the solubility of the soil's organic matter in ammonia is increased if the soil has previously been leached with dilute acid. In order to determine whether or not the method of acid extraction has any influence upon the amount of organic phosphorus obtained in solution, the work recorded in table 7 was performed.

In the experiment, 100-gm. portions of the soil were placed in weighed bottles, and extracted as described:

1. Digested 4 hours with 1000 cc. of HCl to be tenth-normal at the end of digestion, washed free of chlorine with water on a 14-cm. Büchner filter.

TABLE 7

*Data on phosphorus contained in 200-cc. aliquots of ammonia extracts, representing 20 gm. of soil*

METHOD OF REMOVAL OF BASES	PHOSPHORUS		
	Total	Inorganic	Organic
	gm.	gm.	gm.
1. Digested with N/10 HCl, washed Cl free.....	0.0083	0.0012	0.0071
2. Digested with N/5 HCl, washed Cl free.....	0.0080	0.0007	0.0073
3. Digested with N/2 HCl, washed Cl free.....	0.0073	0.0005	0.0068
4. Digested and washed with 1 per cent HCl until practically all soluble removed, washed with water Cl-free.....	0.0079	0.0007	0.0072
5. Like (4), final washing with CO <sub>2</sub> solution.....	0.0079	0.0004	0.0075
6. Like (4), but washing with acid stopped with disappearance of Ca from leachings, washed with water.....	0.0080	0.0008	0.0072
7. Like (6), final washing with CO <sub>2</sub> solution.....	0.0080	0.0003	0.0077
8. Like (6) but washed twice only with 100-cc. portions CO <sub>2</sub> solution.....	0.0084	0.0010	0.0074
9. Digested like (1) washed twice only with water.....	0.0087	0.0016	0.0071
10. Digested like (2) washed like (9).....	0.0085	0.0011	0.0074
11. Washed with formic acid, and water.....	0.0082	0.0016	0.0066
12. Washed with acetic acid, and water.....	0.0053	0.0017	0.0036

2. As before, HCl to be fifth-normal at the end of digestion.

3. As before, HCl to be half-normal at the end of digestion.

4. Digested with 1 per cent HCl, washed with the same until the leachings gave no precipitate with ammonia or ammonium oxalate, washed with water to the absence of Cl from washings.

5. Same as method 4, but final washing with saturated CO<sub>2</sub> solution, as suggested by Beam (1).

6. Same as method 4, except that acid washing stopped when calcium was no longer detected, final washing with water.

7. Like method 6, final washing with saturated CO<sub>2</sub> solution.

8. Like method 6 but washed twice only with saturated CO<sub>2</sub>, 100 cc. each time.

9. Like method 1, washed with two portions of water only, 100 cc. each.
10. Like method 9, except that HCl was to be fifth-normal at the end of digestion.
11. Washed with formic acid equivalent in strength to 1 per cent HCl until leachings were free from calcium, acid washed out with water.
12. Like method 11, acetic acid instead of formic.

After sucking the cakes of soil on the Büchner filters as dry as possible, they were transferred back to the weighed bottles and the proper amount of strong ammonia and sufficient water added to make the weight that of a liter of 4 per cent  $\text{NH}_4\text{OH}$ , the weight of soil constituents removed by the previous acid extractions being allowed for.

The data recorded in table 7 include determinations of total and inorganic phosphorus, and figures for organic phosphorus by difference. It does not appear to make much difference how the extraction is performed, provided hydrochloric acid is used; in so far as any conclusions may be drawn from the slight differences observed, washing with 1 per cent acid until no calcium can be detected in 50 cc. of the leachings and washing the acid from the soil with saturated  $\text{CO}_2$  solution has given the highest figure for organic phosphorus in both cases where this procedure was tested.

In two cases, digestion with N/10 HCl has resulted in a larger amount of total phosphorus but less organic phosphorus being taken into solution by the ammonia than has digestion by fifth-normal acid; in the single case where acid as strong as half-normal was in contact with the soil, the figures for phosphorus are lower, apparently indicating that acid as strong as this may cause solution or decomposition of organic phosphorus compounds.

A small amount of washing of the acid-extracted soil appears to be sufficient; the figures for organic phosphorus are the same, whether care was taken to wash out all chlorine, or whether but two portions of 100 cc. each were used for washing.

Formic acid appears to be less efficient than hydrochloric at equivalent strength; although total phosphorus in the ammonia extract is higher, the figure for organic phosphorus is lower.

Acetic acid is apparently very unsatisfactory.

#### *Other methods for increasing the solubility of the organic matter in ammonia*

The probable presence of a small amount of some organic phosphorus compound in the acid leachings of this soil, indicated both by determinations of total and inorganic phosphorus in acid extracts and by the fact that the acidified ammonia extracts of the soil contain much phosphorus in solution after separating the precipitated organic matter, which cannot be determined as inorganic phosphorus by the usual method, indicate that an efficient method for increasing the solubility of the soil's organic phosphorus in ammonia, avoiding the use of acid, would be desirable.

Fraps and Hamner (8) have described a method for increasing the solubility of humus by the addition of sodium phosphate to the alkali solution used for extraction; this method would not, of course, be suitable for the purpose of the present investigation, but it suggests the use of other precipitants for calcium as additions to the ammonia for this purpose. In table 8 data obtained from analyses of extracts made by the use of ammonium oxalate and of ammonium carbonate, each at the rate of 10 gm. per liter of 4 per cent ammonium hydroxide and 100 gm. of non-acid-extracted soil, are included.

Another procedure which suggested itself was the removal of the bases of the soil by digestion and washing with a solution of ammonium chloride, which would cause a part of the calcium and magnesium of the soil to be replaced by

TABLE 8  
*Constituents of 200 cc. of ammonia extract, representing 20 gm. of soil*

TREATMENT OF SOIL	HUMUS	COLOR	ASH	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	RATIO OF SiO <sub>2</sub> TO Al <sub>2</sub> O <sub>3</sub>	PHOSPHORUS		
								Total	Inor- ganic	Organic
	gm.		gm.	gm.	gm.	gm.		gm.	gm.	gm.
4 per cent NH <sub>4</sub> OH with 1 per cent (NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> ·H <sub>2</sub> O	0.7210*	68	0.0316	0.0015	0.0032	0.0099	0.47	0.0024	0.0010	0.0014
4 per cent NH <sub>4</sub> OH with 1 per cent (NH <sub>4</sub> ) <sub>2</sub> CO <sub>3</sub>	0.5428	50	0.0340	0.0015	0.0025	0.0068	0.60	0.0018	0.0010	0.0008
NH <sub>4</sub> Cl-extracted	0.7808	93	0.0560	0.0048	0.0094	0.0358	0.51	0.0048	0.0014	0.0034
NH <sub>4</sub> Cl-extracted	0.7832	96	0.0480	0.0051	0.0110	0.0134	0.46	0.0050	0.0016	0.0034
HCl-extracted	0.8432	100	0.0712	0.0076	0.0005	0.0596	15.2	0.0086	0.0006	0.0080
HCl-extracted	0.8220	100	0.0564	0.0090	0.0006	0.0162	15.0	0.0080	0.0004	0.0076
HCl in 80 per cent C <sub>2</sub> H <sub>5</sub> OH-extracted	0.6313	70	0.0524	0.0124	0.0029	0.0102	4.28	0.0083		
NH <sub>4</sub> OH only	0.2700	12	0.0236	0.0032	0.0018	0.0006	1.78	0.0011		

\* Determination of oxalate in small aliquots indicated that of 2 gm. of crystallized ammonium oxalate added to each 200 cc., 1.295 gm. remained; this has been deducted to obtain the above figure.

ammonium by interchange and thus removed. The ammonium chloride was afterwards removed by thorough washing with 80 per cent alcohol; neither the salt solution nor this diluted alcohol for washing extracted any color or appreciable amount of organic matter from the soil.

For comparison, there are included the analyses of 4 per cent NH<sub>4</sub>OH extracts made from the same soil after the customary acid extraction, also one made after leaching the soil with 1 per cent hydrochloric acid in 80 per cent alcohol. Finally, to show the small amount of organic matter and phosphorus soluble in pure ammonia unless the soil is previously treated, data obtained from analyses of an extract made by digesting 100 gm. of soil with 1000 cc. of 4 per cent ammonium hydroxide, carbonate-free, are included.

By comparison of the data for the solution mentioned last with data obtained from analyses of solutions prepared in the same way except that additions of 10 gm. per liter of ammonium oxalate and carbonate were made to the ammonia used for extracting the soil, it is seen that the additions named have been quite effective in increasing the solubility of the soil constituents in ammonia although not to nearly so great an extent as the regular acid extraction. The oxalate has been more effective than the carbonate, as indeed might be expected from the slighter solubility of the corresponding calcium compound.

The ammonium chloride extraction has apparently been very effective in increasing the amounts of total organic matter and color taken into solution; it is not, however, proportionately effective in increasing the solubility of the organic phosphorus.

The ammonia extract of the sample which was previously extracted by 1 per cent hydrochloric acid in 80 per cent alcohol is quite low in total organic matter and color. The failure of the ammonia to extract more organic matter and color is attributable rather to previous removal of these constituents by the

TABLE 9  
*Calcium and magnesium extracted from soil by methods described*

MANNER OF EXTRACTION	CALCIUM	MAGNESIUM
	<i>per cent</i>	<i>per cent</i>
Digestion and washing, 1 per cent HCl afterwards washed with water ..	0.6105	0.0704
Digestion and washing, normal $\text{NH}_4\text{Cl}$ , afterwards washed with 80 per cent alcohol.....	0.5280	0.0482
Digestion and washing with 1 per cent HCl in 80 per cent alcohol, washed with 80 per cent alcohol.....	0.6080	0.0530

acid-alcohol than to poor removal of bases in the preliminary leachings. The amounts of calcium and magnesium extracted from 100 gm. of this soil by the several methods of extraction described are shown in table 9.

Similarly, it seems probable that the comparatively large amount of organic matter and color extracted from the soil previously leached with ammonium chloride solution is at least partly due to the fact that here practically no color and very little organic matter was removed from the soil during the preliminary leaching; even 1 per cent hydrochloric acid in aqueous solution removes more organic matter than would be desirable if the amount of "humus" in the ammonia solution were the only consideration.

The solutions which were prepared without the use of acid for preliminary extraction of bases are without exception low in silica, but contain more alumina, so that the ratio of silica to alumina is invariably less than 2.1, indicating that the alumina is in excess and that a part of it is present in a form other than clay, perhaps simply dissolved by the ammonia.

The amount of ferric oxide seems to follow to some extent other constituents soluble in ammonia; the very large amount present in one of each pair of solu-

tions prepared by acid and ammonium chloride extraction is an illustration of the variability of this constituent due to very slight differences in manipulation, these solutions not having been prepared at the same time as the others.

The content of inorganic phosphorus is highest in those solutions made without previous acid treatment; it is probable that leaching with hydrochloric acid reduced the content of inorganic phosphorus in the ammonia extracts by removing inorganic phosphorus capable of going into solution in ammonia. Some of the data in table 13 lend support to this view.

None of the substitutes for acid extraction can be considered successful for increasing the solubility of organic phosphorus in the case of this basic soil; the best is less than 50 per cent effective as compared with acid extraction.

*Use of alkalies other than ammonia as solvents for organic phosphorus*

As was recently demonstrated by Gortner (9), there may exist wide differences between the solvent effects of different alkalies, or different concentrations

TABLE 10

*Constituents of 200 cc. of alkali extracts, representing 20 gm. of acid-extracted soil*

ALKALI	PHOSPHORUS			COLOR
	Total	Inorganic	Organic	
	gm.	gm.	gm.	
Normal KOH.....	0.0094			65
N/4 KOH.....	0.0104	0.0014	0.0090	67
Normal NaOH.....	0.0106			79
N/4 NaOH.....	0.0107	0.0015	0.0092	86
Normal LiOH.....	0.0098			96
N/4 LiOH.....	0.0106	0.0016	0.0090	83
Normal Na <sub>2</sub> CO <sub>3</sub> .....	0.0082			65
Normal HNaCO <sub>3</sub> saturated with CO <sub>2</sub> .....	0.0028			22
N/4 HNaCO <sub>3</sub> saturated with CO <sub>2</sub> .....	0.0011			20
Normal NH <sub>4</sub> OH.....	0.0092	0.0001	0.0091	100

of the same alkali, upon the organic matter of the soil. The alkaline solutions enumerated in table 10 were employed as solvents for the alkali-soluble phosphorus of this soil, 100-gm. portions of the acid-extracted and dried soil being shaken during one working day with 1 liter of the several solutions and filtered on 25-cm. Büchner funnels.

Aliquots of 200 cc. were employed for determinations of total and inorganic phosphorus, the methods previously described being used, with such modifications as were necessitated by the large amounts of fixed alkali present in some cases.

In the cases of all the fixed alkalies, the fourth-normal solutions contained more total phosphorus than the normal solutions, indicating that the organic phosphorus is less soluble in the more concentrated solutions; in the case of sodium hydroxide the difference is too small to be considered, and this case



may be an exception. Normal sodium carbonate has a moderate solvent action, but the bicarbonate solutions saturated with  $\text{CO}_2$  were much less efficient.

At the fourth-normal concentration, the hydroxides of the alkali metals caused more total phosphorus to be taken into solution than did normal ammonium hydroxide, but determinations of inorganic phosphorus show that as solvents for organic phosphorus, they cannot be considered superior to ammonia.

Ammonia is also the best solvent for color, although this may be due in part to the fact that the ammonia solution contained by far the greatest amount of iron, the stronger alkali solutions containing very little.

The solutions of the fixed alkalies all dissolved large amounts of silica and alumina from the soil.

Among the mixtures of alkaline hydroxide and soil, striking differences were observed in behavior on standing; the suspensions containing potassium hydroxide coagulated and settled, and were easily filtered; those containing lithium hydroxide coagulated, but settled to only a slight extent and were filtered with difficulty. Sodium hydroxide occupies an intermediate position between these extremes, while the suspension of soil in ammonia neither coagulated nor settled, and next to the fourth-normal lithium hydroxide was the most difficult to filter.

#### *Completeness of extraction by one treatment*

Fraps (5) and, more recently, Russell and Prescott (17) have studied the solubility of the inorganic phosphorus of the soil in dilute acids; they are agreed that the quantity of phosphorus taken into solution at the first treatment is not the total amount capable of being dissolved from the soil by the particular dilute acid employed as a solvent, but is the difference between that quantity and the phosphorus again fixed or absorbed from the solution by the soil. As the disturbing effect of this absorption is undoubtedly one of the great difficulties encountered in studies of the probable availability of the soil's natural supply of phosphorus based upon solubility in dilute acids, it will be of interest to know whether or not any similar effect is operative to prevent the complete extraction of the organic phosphorus of the soil by a single treatment with ammonia.

The plan adopted for the determination of this point consisted of making successive extractions of the same portions of soil, weighing the bottles and filtration apparatus at appropriate times in order to get the necessary data to correct for volume of the preceding extract left in the cake of soil on the filter and for any change in volume due to evaporation.

A quantity of acid-extracted soil from "lot 2" was weighed into each of two liter bottles, and the proper amount of ammonia solution added to make exactly 1 liter of 4 per cent  $\text{NH}_4\text{OH}$  in contact with 100 gm. of moisture-free acid-extracted soil. The mixture was shaken 8 hours and filtered on a

25-cm. Büchner funnel without any precipitant, the funnel being covered with a well-fitting glass plate. The cake of soil remaining on the filter was replaced in the bottle, again made to the proper weight with 4 per cent  $\text{NH}_4\text{OH}$ , allowing for matter extracted, and the process repeated.

In all, four successive extracts of the duplicate portions of soil, designated A and B, were thus obtained; the amount of each preceding extract mixed with the next was found to be 8 per cent. The error by evaporation was less than 1 per cent, and is included in the above correction.

In table 11 the data obtained from this experiment are presented.

The results indicate that each successive extraction has removed a slight additional amount of every soil constituent determined, allowance being made for the 8 per cent of the previous extract mixed with those after the first. The data for organic phosphorus only will be discussed in detail; the

TABLE 11

*Constituents of 200 cc. of 4 per cent  $\text{NH}_4\text{OH}$  extract, representing 20 gm. of moisture-free acid-extracted soil*

EXTRACT AND SAMPLE	HUMUS	COLOR	ASH	$\text{SiO}_2$	$\text{Al}_2\text{O}_3$	$\text{Fe}_2\text{O}_3$	PHOSPHORUS		
							Total	Inorganic	Organic
	gm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.
First { A.....	0.7798	107	0.1017	0.0331	0.0038	0.0264	0.0091	0.0004	0.0087
{ B.....	0.7922	107	0.1152	0.0420	0.0040	0.0257	0.0091	0.0003	0.0088
Second { A.....	0.1612	12	0.0441	0.0209	0.0027	0.0045	0.0012	0.0001	0.0011
{ B.....	0.1501	11	0.0294	0.0131	0.0005	0.0031	0.0010	0.0002	0.0008
Third { A.....	0.0642	3	0.0224	0.0100	0.0006	0.0013	0.0004	0.0002	0.0002
{ B.....	0.0836	4	0.0247	0.0096	0.0008	0.0026	0.0004	0.0002	0.0002
Fourth { A.....	0.0508	2	0.0249	0.0106	0.0007	0.0009	0.0002	0.0001	0.0001
{ B.....	0.0476	2	0.0232	0.0112	0.0005	0.0010	0.0002	0.0002	

second extracts vary more in their composition than is desirable, that from sample A being considerably higher in ash constituents than that from sample B, indicating that the filtration in this case was probably less effective. If the data for the second extract of sample A are used as a basis for calculations, the second extraction has brought into solution approximately 0.4 mgm. of organic phosphorus; if the data for the second extract B are used, then only about 0.1 mgm. has been brought into solution. Analyses of the third and fourth extracts indicate that not more than 0.1 mgm. of organic phosphorus has been brought into solution by each extraction.

Of a total of 9.3 mgm. and 9.0 mgm., respectively, for samples A and B brought into solution by four extractions, 8.7 and 8.8 mgm., or 94 and 98 per cent were contained in the first extract, indicating that the organic phosphorus contained in the first ammonia extract approximates the total amount which may be brought into solution by ammonia.

*Organic phosphorus in acid leachings*

Potter and Benton (15) found that the acid leachings of the soils investigated were free from organic phosphorus. In view of the importance of the question, it was considered advisable to determine whether this was also the case with the soil under investigation by the writer; accordingly, 500-cc. aliquots of the 1 per cent HCl leachings and water washings of this soil combined were precipitated by 50 cc. of magnesia mixture, and one-tenth volume concentrated ammonia after the addition of 10 gm. of crystallized tartaric acid to hold iron and aluminum in solution. After standing 3 days, the precipitate was filtered off, thoroughly washed, redissolved in dilute  $\text{HNO}_3$  and precipitated by acid molybdate, the determination being finished in the usual manner.

The percentage of recovery by this method varied from 95 to 97, somewhat less than a centigram of total phosphorus being present in the 500-cc. aliquots of the several acid solutions examined.

A synthetic solution was prepared, corresponding as closely as possible in iron, aluminum, calcium, magnesium and phosphorus content to one of the acid extracts under investigation; by the same method, 99 per cent of the phosphorus content was recovered. The absolute amounts of phosphorus not determined as inorganic in these solutions are so small, however, that the organic phosphorus in the acid extracts of this soil may be considered a negligible quantity.

*Nature of the ammonia-soluble phosphorus of the soil*

In the preceding discussions of the phosphorus contained in these alkali extracts of soil, the term "organic phosphorus" has repeatedly been used, when the phosphorus in solution not present as the orthophosphate ion or determinable by the usual method for inorganic phosphorus, was meant. In a few cases, where the humus ash was unusually high and had a composition indicating the probable presence of considerable clay, the term "organic phosphorus" has been qualified by the statement that probably a little inorganic phosphorus contained in clay was included.

Efforts to learn the identity of the organic phosphorus compounds in the ammonia extract of this soil have so far been without success; when the ammoniacal extract is acidified with acetic acid and a small amount of picric acid added to precipitate proteins, as described by Levene (13), a brownish black precipitate is obtained, which was found to contain 7.5 per cent of the total phosphorus in the original humus solution. To the clear deep reddish brown liquid somewhat more than its own volume of 95 per cent alcohol was added and the mixture allowed to stand; the dark-colored voluminous precipitate which separated contained 37.8 per cent of the total phosphorus originally present. Repeated solution of this precipitate in ammonia and reprecipitation by acetic acid and alcohol did not lighten its color appreciably; when boiled under a reflux condenser with 10 per cent sulfuric acid and the tests

for the decomposition products of nucleic acid applied as described by Schreiner and Lathrop (19), orthophosphoric acid was the only one which could be positively identified. At almost all stages of these separations, voluminous gummy precipitates appeared, highly colored in most cases.

The method for separating the organic phosphorus compounds from ammonia extracts described by Jegorev (12) proved entirely useless in the present case.

In the absence, then, of any direct proof that all the phosphorus in ammonia extracts not determined as inorganic phosphorus is actually in organic combination, it will be necessary to consider all the other possible explanations of its state of combination.

The fact that added phosphate is completely recoverable is proof that the phosphorus not determined by the method for inorganic phosphorus is not inorganic phosphorus prevented from precipitating by any occult influence attributable to other constituents of the solution.

It has likewise been shown that it is possible to obtain these ammonia extracts with a high content of organic phosphorus but practically free from clay; this is proof that phosphorus enclosed in mineral particles and so protected from attack by dilute acid is not concerned.

Fraps (7), in his discussions of the nature of the phosphorus in the ammonia extract of soil, has directed attention to the surprising solubilities of the phosphates of iron and aluminum in ammonia and their relatively difficult solubility in dilute acids. Following additions of these phosphates to ammonia, and to an ammoniacal soil extract, it was found that in each case a considerable amount of the phosphate had been taken into solution, as shown by increases in phosphorus and iron or aluminum content. Determinations of inorganic phosphorus were made; it was found that the phosphorus thus added was completely recoverable as inorganic phosphorus.

Gortner and Shaw (10) offer as an explanation for the presence of phosphorus in a form not determinable by the method for inorganic phosphorus, the theory that phosphoric acid is adsorbed by colloidal organic matter from acid leachings during the preliminary extraction of bases, and being held in the adsorbed state after the organic matter has been removed from the soil by ammonia is thus included with the organic phosphorus, or if not so held is possibly again adsorbed by organic matter as soon as the precipitated magnesium ammonium phosphate and organic matter obtained by a magnesia mixture precipitation of an ammoniacal soil extract is made acid in leaching out the inorganic phosphorus. As evidence opposed to this theory, attention is directed to the fact that added phosphate is recovered quantitatively as inorganic phosphorus irrespective of the actual amount added, within wide limits, and with a constant increment corresponding to the solution's original content of inorganic phosphorus. If adsorption is a factor of importance in the present connection, the figure representing the original content of inorganic phosphorus in the solution would not remain a constant with variations

in the amounts of phosphate since concentration of the substance adsorbed in the solution is one of the factors governing the amount of the substance removed from the solution by an adsorbent.

This statement is made with knowledge of the results reported by Prescott (16) for adsorption of phosphoric acid from N/20  $\text{HNO}_3$  by precipitated humus, in which the amount of  $\text{P}_2\text{O}_5$  adsorbed shows no consistent relation to the concentration of this constituent and in fact is nearly a constant. Prescott neither considers the possibility of the presence of organic phosphorus in his humus preparation, nor the possibility of chemical precipitations by constituents of the humus solution; in either event, the amount of phosphorus precipitated on acidifying might be almost constant, regardless of the amount of  $\text{P}_2\text{O}_5$  added.

Further evidence that the phosphorus of alkali extracts of soil not determinable as inorganic phosphorus is not adsorbed by humus or mineral colloids is found in the fact that upon acidifying the alkali extract, only a part of the phosphorus is precipitated, but determinations of inorganic phosphorus in the clear solution separated show even less inorganic phosphorus than was originally in the solution. As has been mentioned, acidification with acetic acid caused 7.5 per cent of the total phosphorus to be precipitated; hydrochloric acid precipitated a much larger amount, 44 per cent in one case. As acidifying with hydrochloric acid causes a larger precipitate of organic matter than is produced by the use of acetic acid, it was thought that repeated solution in ammonia and reprecipitation by hydrochloric acid might bring more phosphorus into solution; four repetitions of this treatment reduced the amount in the precipitate to 40 per cent of the total phosphorus present.

A 1 per cent sodium hydroxide extract of the same soil, the organic phosphorus content of which was about the same as that of the ammonia extracts described, about 9 mgm. per 200 cc., was acidified with hydrochloric acid; the phosphorus content of the precipitate was 14 per cent of the total amount originally in solution.

Determinations of inorganic phosphorus in these acid filtrates indicated no appreciable decomposition of the organic phosphorus compounds by the treatment.

The data discussed were obtained from work on humus solutions prepared in the customary way; in some cases, phosphorus was added to the finished solution, before the analyses were made. The results obtained from ammonia extracts prepared from soil to which phosphorus had been added before the alkaline extraction was begun should be of interest, because in this case the phosphorus is undoubtedly adsorbed in part under conditions similar to those postulated by Gortner and Shaw. Two cases present themselves:

a. Phosphorus is absorbed from neutral or alkaline solution; here the absorption may possibly include chemical precipitation as well as adsorption.

b. Phosphorus is adsorbed from acid solution and acid with excess of phosphorus removed by washing with water.

a. Both acid-extracted and unextracted soil were used for this experiment; two 100-gm. portions were placed in bottles, 1000 cc. of 4 per cent  $\text{NH}_4\text{OH}$  added to one bottle of each pair and 1000 cc. of 4 per cent  $\text{NH}_4\text{OH}$  containing ammonium phosphate equivalent to 10.5 mgm. of phosphorus added to the other bottles. The mixtures were shaken at intervals for several weeks, when 10 gm. of powdered ammonium carbonate was added to each bottle, the contents well shaken and transferred to centrifuge bottles. The centrifuged extracts were free from clay; data obtained from determinations of total and inorganic phosphorus are presented in table 12.

From the data in table 12, it appears that of the 2.1 mgm. of phosphorus added to each 200 cc. of solution, only 0.4 and 0.6 mgm. as shown by the determination of total and inorganic phosphorus, respectively, remain after contact with the unextracted soil. The unextracted soil is able to remove from the alkaline solution about 75 per cent of the added phosphorus in this case; the acid-extracted sample under similar circumstances apparently possesses no power of fixation, as the excess by both total and inorganic determinations

TABLE 12

*Phosphorus in 200 cc. of 4 per cent  $\text{NH}_4\text{OH}$  extract, representing 20 gm. of soil*

SAMPLE	TOTAL	INORGANIC	ORGANIC
	mgm.	mgm.	mgm.
Unextracted, check.....	1.8	0.2	1.6
Unextracted, phosphorus added.....	2.2	0.8	1.4
Acid-extracted, check.....	6.9	0.1	6.8
Acid-extracted, phosphorus added.....	9.0	2.2	6.8

corresponds to the amount added. The presence of inorganic phosphorus in the solution at the moment of extraction by alkali causes no increase in the figure for organic phosphorus.

b. The soil for this experiment had been acid-extracted and dried; two 100-gm. portions were shaken with 1000 cc. of  $\text{N}/5 \text{ HNO}_3$ , and two portions with the same reagent containing, in each 200 cc., 5 mgm. of phosphorus in the form of ammonium phosphate. The mixtures were mechanically shaken for half a day, let stand over night, filtered on 14-cm. Büchner funnels and the cakes of soil washed with somewhat less than a liter of water, which was ample for the removal of soluble acid. The filtrate and washings were made to 2000-cc. and 400-cc. aliquots removed for determinations of total phosphorus, which aliquots correspond to 20 gm. of the sample.

The cakes of soil in the Büchner filters were transferred back into the bottles and sufficient ammonia and water added to make the volume 1000 cc. and the strength 4 per cent  $\text{NH}_4\text{OH}$  in contact with the 100 gm. of soil. These mixtures were mechanically shaken for the greater part of two days and finally filtered on 25-cm. Büchner funnels without the use of any coagulant. Corresponding portions from the same lot of acid-extracted soil, but not sub-

jected to the second extraction by N/5  $\text{HNO}_3$ , were extracted by ammonia in the same way and at the same time.

The data obtained from this experiment are presented in table 13.

Fifth-normal nitric acid followed by water washing was able to extract 1.1 mgm. of phosphorus from each 20 gm. of the soil which had already been once extracted. The addition of 5.0 mgm. of phosphorus to the nitric acid did not raise the phosphorus content to 6.1 mgm. after contact with the soil and addition of washings; only 5.3 mgm. was found, indicating that 0.8 mgm. of phosphorus was held by the soil in such a way that neither digestion with dilute acid containing some phosphorus nor a reasonable amount of washing with water was able to remove it. If adsorption really is the factor responsible for the retention of phosphorus in cases such as this, as claimed by Russell and Prescott (17), then one is justified in the conclusion that this phosphorus is adsorbed. Ammonia extractions of the residues of soil following these second acid treatments afford data indicating that this adsorbed phosphorus is completely extracted by ammonia and such ammonia-soluble phosphorus is determinable as inorganic phosphorus.

TABLE 13  
*Phosphorus contained in extract representing 20 gm. of soil*

METHOD OF EXTRACTION	TOTAL	INORGANIC	ORGANIC
	mgm.	mgm.	mgm.
(A) N/5 $\text{HNO}_3$ .....	1.1		
(B) N/5 $\text{HNO}_3$ containing 5.0 mgm. P.....	5.3		
4 per cent $\text{NH}_4\text{OH}$ extract of residue A.....	9.4	0.7	8.7
4 per cent $\text{NH}_4\text{OH}$ extract of residue B.....	10.3	1.8	8.5
4 per cent $\text{NH}_4\text{OH}$ .....	9.8	1.1	8.7

*Composition of ammonia extracts of four depths of soil*

Ammonia extracts of the four depths of the Paulding soil were prepared; 100-gm. samples were digested with 500 cc. of 1 per cent  $\text{HCl}$  for 4 hours with frequent shaking, filtered and washed on 13-cm. Büchner funnels with 1 per cent  $\text{HCl}$  until leachings were free from calcium, and finally washed with saturated  $\text{CO}_2$  solution until chlorine-free. The cakes of soil were placed in bottles and water and strong ammonia added to make the strength 2.5 per cent  $\text{NH}_3$  and the volume of solution in contact with the soil 1000 cc.; the mixtures were shaken 8 hours in a machine and finally filtered on 25-cm. Büchner funnels without the addition of any precipitant. The composition of the extracts is shown in table 14; no analyses of the humus ash were made. In table 15, the humus content, color and organic phosphorus content are presented in the form of ratios. The close correspondence of color, humus content and organic phosphorus are noteworthy and indicate that the ammonia-soluble organic matter of this soil has a very similar composition in all the depths sampled. Furthermore, the total nitrogen content of the four depths of the soil stands in similar ratio.

TABLE 14

*Constituents of 200 cc. of 2.5 per cent  $NH_3$  extract, representing 20 gm. of soil*

DEPTH	HUMUS	ASH	PHOSPHORUS		
			Total	Inorganic	Organic
<i>inches</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
0-6	0.6588	0.0596	0.0077	0.0009	0.0068
6-12	0.4132	0.0648	0.0062	0.0010	0.0052
12-18	0.2920	0.0740	0.0052	0.0015	0.0037
18-24	0.2068	0.0636	0.0039	0.0015	0.0024

TABLE 15

*Ratios of humus, color and organic phosphorus in ammonia extracts, and total nitrogen in soil*

DEPTH	HUMUS	COLOR	ORGANIC PHOSPHORUS	NITROGEN IN SOIL
<i>inches</i>				
0-6	100	100	100	100
6-12	63	62	76	66
12-18	44	44	54	51
18-24	31	33	35	37

*Organic phosphorus indicated by other data*

Various investigators have considered the increase in the amount of phosphorus dissolved from a soil by acid extraction following ignition to represent organic phosphorus; while Fraps (6) has shown that this is not necessarily true, it will be of interest to note the effect of ignition upon the solubility of the phosphorus in the four depths of the soil under consideration. Twenty-gram portions of soil were heated in a muffle at about 500°C. for one-half hour; similar portions of unignited soil were used as checks; the method adopted for extracting the soluble phosphorus consisted of an overnight digestion with 200 cc. of cold 2 per cent HCl, followed by filtration on a 10-cm. Büchner funnel and washing with cold 1 per cent HCl to a total volume of 800 cc. Phosphorus was determined in the entire filtrate by the usual method after evaporating with nitric acid to expel chlorine.

The acid employed for the experiment described is very dilute in comparison with the 12 per cent acid employed for a similar purpose by Stewart (20); it was thought better to use the more dilute acid in order to avoid solution of organic phosphorus from the unignited samples. The procedure of washing with the acid following digestion instead of digestion alone, was intended to reduce to the minimum the amount of adsorbed phosphorus remaining in the extracted soil.

The data obtained from determinations of phosphorus in these extracts of the unignited and ignited samples are presented in table 16; it will be ob-



served that the quantities of phosphorus extracted from the unignited samples in the three lower depths are very similar, and that the same is true of all four depths after ignition. The differences observed do not indicate that results obtained from determinations of ignition-soluble phosphorus would throw any light upon the amounts of organic phosphorus in this soil.

By reference to table 1, it will be seen that the total potassium content of the four depths of this soil is quite uniform; the soil and subsoil may therefore be considered to be of practically the same mineral composition, and the method for calculating the content of organic phosphorus proposed by Hopkins and Pettit (11), may be applied. The results obtained are considerably at variance from the indications afforded by analyses of ammonia extracts of the corresponding samples from the lower depths, although the correspondence for the first depth is very close; by deducting from the total phosphorus content of the lowest depth the amount of phosphorus indicated to be organic by analyses of the ammonia extract, and using the amended figure for mineral phosphorus as the base, the results may be expected to be somewhat

TABLE 16  
*Phosphorus extracted from 20 gm. of soil, not ignited and ignited*

DEPTH	NOT IGNITED	IGNITED	DIFFERENCE
<i>inches</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
0-6	7.7	9.4	1.7
6-12	6.3	9.3	3.0
12-18	6.2	9.2	3.0
18-24	6.0	8.7	2.7

better. This was done in obtaining the figures for organic phosphorus by the calculations given in table 17, last column.

The figures for organic phosphorus in the two intermediate depths now show satisfactory agreement with those obtained from analyses of the ammonia extract, but indications for the surface sample are too high: Several explanations for this are possible:

1. Extraction of organic phosphorus by ammonia has been incomplete; this seems unlikely in view of the apparently satisfactory extraction in the case of the lower depths.

2. The surface layer is not the same in mineral composition as the samples taken at lower depths; this may be a partial explanation, as the total potassium content of the surface layer shows the greatest departure from the mean.

3. The calculation method, in addition to the first incorrect assumption that the subsoil is free from organic phosphorus, involves another, namely, that organic phosphorus, once formed, does not again revert to the inorganic state. It is quite possible that as much phosphorus as is indicated by the calculation method, as corrected, has once been in the organic state, although no more remains organically combined than is obtained in ammonia solution.

4. Gortner and Shaw call attention to the fact that not all the phosphorus of plants is organic, since potassium dihydrogen phosphate is present in many vegetable saps. There would thus be a concentration of inorganic phosphorus in the surface layer of the soil through the action of the plant in addition to the accumulation of organic phosphorus. In this connection, it should be noted that such is apparently the case, as shown by the larger amount of acid-soluble phosphorus in the surface soil, although the factors enumerated under subjects 2 and 3 above may apply here also.

TABLE 17

*Organic phosphorus in soil, by several methods*

DEPTH	NH <sub>4</sub> OH-SOLUBLE ORGANIC	IGNITION-SOLUBLE ORGANIC	CALCULATED	
			Hopkins-Pettit	Corrected
<i>inches</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0-6	0.034	0.009	0.036	0.048
6-12	0.026	0.015	0.016	0.028
12-18	0.019	0.015	0.004	0.016
18-24	0.012	0.014		(0.012)

#### SUMMARY

In this paper, analytical methods adapted to the determination of total and inorganic phosphorus in ammonia extracts of soils are described.

A satisfactory method for separating clay from ammoniacal soil extracts, having in view the maximum content of organic phosphorus, has been determined.

The proper procedures and conditions for the preliminary removal of bases from the soil and extraction by ammonia solution in the preparation of ammoniacal extracts intended for the study of the soil's content of organic phosphorus have been determined.

It is shown that as solvents for the organic phosphorus of the soil studied, solutions of the hydroxides of the fixed alkalis are not superior to ammonia. One extraction by ammonia, following the proper procedure, is shown to remove practically all the organic phosphorus from the soil that is capable of being taken into solution by ammonia.

No consistent relations between the contents of ammonia-soluble organic matter (humus), humus ash, silica, ferric oxide and alumina in these solutions could be observed. No constant relation between total organic matter and organic phosphorus was observed in ammonia extracts prepared in various ways, although there was a general tendency for these to vary together.

Evidence is presented that inorganic phosphorus adsorbed by colloids, organic or inorganic, is not included in the apparent content of organic phosphorus, as determined by the methods described.

The data obtained indicate that the organic phosphorus, as determined from analyses of properly-made ammonia extracts, approximates to the probable content of organic phosphorus in the four depths of this soil sampled.

Determinations of humus, color and organic phosphorus in ammonia extracts of four depths of the soil indicate that these ammonia soluble constituents are present in about the same relative proportions in the four depths examined. The total nitrogen contents of the four depths of soil stand in ratios very similar to those exhibited by the ammonia-soluble constituents named.

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## CROSS-INOCULATION OF LEGUMES

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### INTRODUCTION

The work reported on in this short paper deals with the cross-inoculation of the various members of the common legumes. The extent to which various legumes will cross-inoculate is a very practical problem. For instance, if the same organisms will cause the formation of nodules on all the clovers but one culture containing but one strain of organism will be necessary to infect the seeds, when a mixture of the seeds of such legumes as crimson, red, and white clover is planted. If cross-inoculation did not take place, three separate and distinct organisms would be necessary to produce the same results.

As early as 1904, Hopkins (4) appreciated the fact that a very great similarity existed between the nodules of two different plants. At that time, he demonstrated the fact that the nodule production and growth of alfalfa was very much the same as that of sweet clover. He also showed that the bacteria taken from the roots of sweet clover caused the formation of nodules on the alfalfa plant as well as did the organisms taken from the roots of alfalfa.

Garman and Didlake (2) in 1914 reported the results of an extensive work in determining to what extent the organisms of the various legumes would cross-inoculate. They concluded that the organisms which cause the formation of nodules on the roots of various legumes may be grouped under 6 different species.

From the results of their work in which they tested the extent to which the legumes will cross-inoculate, Burrill and Hansen (1) show that the nodule organisms are divided into 11 distinct groups. These investigators include the 6 different species as given by Garman and Didlake (2) as 6 of their groups, and add 5 more.

From the practical standpoint, in the cross-inoculation of legumes, three very important questions present themselves. These are:

1. Will the organisms causing the production of the nodules on the particular plants always infect all the members of that group?
2. Is the nodule production and the size and vigor of the plant, the roots of which produced nodules caused by the cross-inoculation, as vigorous and strong, respectively, as that produced by the inoculant of the plant by the organism of its own kind (that which is isolated from that specific plant)?

3. Will the organisms cause nodule production by cross-inoculation after the culture has been in storage, without transfers having been made continuously at regular intervals, as for instance, if cultures were kept in storage on an agar medium, as under commercial methods? Hence, how long a time will these organisms (by cross-inoculation) retain their vigor of causing nodule production? The first two questions named above will be considered in the present paper.

#### METHODS AND MATERIALS

##### *Culture media*

The culture medium upon which the various *Bacillus radicola* were isolated and grown, was that which was perfected by the senior author<sup>1</sup> for the Mulford Biological Laboratories. This proved very satisfactory for a luxuriant as well as a rapid growth for most of the various species of the legume organism.

##### *Isolation of organisms*

The method for isolating the organisms from the nodules of the various legume plants was similar to the one employed by Harrison and Barlow (3). This was as follows: By means of small forceps, several medium-sized nodules were taken from the roots of a young plant and placed in a sterile petri dish. The nodules were washed very carefully several times with sterile water. After this washing, they were allowed to remain in bichloride-hydrochloric acid solution (1) for from two to four minutes, after which they were soaked in sterile water for four minutes. The sterilizing solution was then washed off with two more washings of sterile nitrogen-free solution. By means of a sterile scalpel the nodules were crushed on a sterile (flamed) slide. A loopful of this cloudy suspension was transferred to a tube of agar that had been liquefied (temperature of 43°C.). Several transfers were made from this tube to other liquefied agar tubes, several dilutions thus being made. The contents of these tubes were poured into petri dishes. After a few days, when individual colonies had appeared on the plates, transfers from these were made to agar slants. After these cultures had grown on the agar slants, they were carefully examined in Smith tubes and under a microscope for contaminations. If free from contaminating organisms, they were ready for use. If contaminated, they were plated out again.

All of the legume organisms herein considered were isolated from the roots of the plants by the above method.

<sup>1</sup> Koch, Geo. P. Comparison of various culture media for *B. radicola* (Not yet in print.)

*Plant tests*

For testing nodule production on plants, the agar test-tube method, employed by Garman and Didlake (2), was tried, and proved quite successful. By this method, however, conditions, being for the most part anaerobic, would be much more unnatural than the conditions under which these organisms under ordinary growth and development usually exist. Hence, a method whereby the conditions were as natural as possible, was worked out and employed. This method which proved very successful for our plant tests was as follows: Earthenware pots, of  $3\frac{1}{2}$  and  $4\frac{1}{2}$  inch inside diameter, were filled with fine sand, to which the following inorganic salts were added: 12.5 gm. calcium carbonate, 10.0 gm. calcium phosphate, 5.0 gm. potassium sulfate and 1.3 gm. magnesium sulfate per 25 kgm. After the sand in the pots was saturated with water, the pots were carefully wrapped in heavy paper and sterilized in the autoclave for 2 hours at 15 pounds pressure on 2 successive days.

The pots, having been sterilized, were planted with sterile seed. The method of sterilizing the seeds was as follows: They were placed in a sterile wide-mouthed bottle and then soaked in bichloride-hydrochloric acid solution for from 3 to 7 minutes, after which time the sterilizing solution was washed from the seed. The seeds were then allowed to soak in sterile water for the same length of time that they were in the sterilizing solution, and were then washed three times in sterile water. The seeds were next planted with sterile platinum tipped forceps, those of the larger legumes being planted in the  $4\frac{1}{2}$ -inch pots, while those of the smaller seeds were planted in the  $3\frac{1}{2}$ -inch pots. After planting, the pots were carefully placed on sterile glass plates in the greenhouse and covered with sterile bell jars.

The seeds were infected soon after planting. These were infected by carefully washing the organisms from the cultural growth on agar slants with 7 cc. of nitrogen-free solution. This suspension of organisms was then transferred to the pots by means of sterile pipettes. After the plants had grown from 3 to 4 weeks, they were taken from the pots, the sand washed from the roots, and the roots very carefully examined for nodulation. Each determination (treatment) was made in duplicate or triplicate (two or three pots). Several series of controls, (pots with seeds not inoculated), were always made. If correct technique was carried out and they were free from contaminations the roots of the control plants which were not infected, should have had no nodules.

Each experiment embodying the cross-inoculation of each group of organisms was carried out at least three times, and the results herein reported are the final average of the several experiments.



## RESULTS

*Alfalfa group*

According to previous investigators, this group comprises the plants of *Medicago*, *Melilotus* and *Trigonella foenum-graecum*. Since alfalfa, sweet clover and burr clover are the most common and probably the only legumes of this group that are of any practical importance, these were the only ones considered in this work.

TABLE 1

*The results of the cross-inoculation of B. radiculicola of the alfalfa group*

KIND OF PLANT	RESULTS OF THE INOCULATION			
	Not inoculated	Alfalfa	Sweet clover	Burr clover
Alfalfa.....	— *	3+	3+	3+
Sweet clover.....	—	3+	3+	2+
Burr clover.....	—	1+	1+	2+

\* Throughout this work (—) indicates no nodule production, (1+) fair nodule production, (2+) good nodule production, (3+) very vigorous nodule production.

The results, as shown in table 1 above, demonstrate the fact that each organism isolated from the particular plant of this group of legumes caused the formation of nodules on the other members of the group, as it inoculated its original host. There is, however, a rather marked difference in the extent to which the organisms isolated from the various plants cause such nodulation. It is apparent that the alfalfa and sweet clover plants were strongly inoculated by the organisms of all these plants. The burr clover plants produced very few nodules when inoculated with the alfalfa and sweet clover organism. In fact, several plants had no nodules at all.

*Clovers (genus Trifolium)*

This group comprises all the clovers of the genus *Trifolium*, namely: mammoth red, alsike, crimson, red, white and zigzag clover. But 4 of the most commonly grown were studied in these experiments; these were crimson, alsike, red, and white. The results of the experiments are shown in the table below.

The results as presented in table 2 demonstrate conclusively, that the *radiculicola* organism of these 4 clovers of the genus *Trifolium* cross-inoculated very well, and the nodule production, resulting from the infection produced by the cross inoculation, was as vigorous on each plant as in cases where the infection was produced by its own organism. The white clover plants, for instance, when inoculated with organisms isolated from the red clover had as many and as large nodules, and the plants were as vigorous as when the inoculation was made with the organism isolated from the white clover plants.

TABLE 2

*The results of the cross-inoculation of B. radiculicola of the trifolium clovers*

KIND OF PLANT	RESULTS OF THE INOCULATION				
	Not inoculated	Crimson	Alsike	Red*	White
Crimson.....	—	2+	2+	2+	3+
Alsike.....	—	3+	3+	3+	3+
Red.....	—	3+	3+	3+	3+
White.....	—	3+	3+	3+	3+

*Pea-vetch group*

This group represents plants of genii *Pisum*, *Vicia*, *Lathyrus* and *Lens*. Of this group, members of each genus which represented the common plants under cultivation, were employed in this work. These were vetch, sweet pea, Canada field pea and garden pea.

TABLE 3

*Showing the results of the cross-inoculation of B. radiculicola of the pea-vetch group*

KIND OF PLANT	RESULTS OF THE INOCULATION				
	Not inoculated	Vetch	Sweet pea	Canada field pea	Garden pea
Vetch.....	—	3+	2+	2+	3+
Sweet pea.....	—	3+	3+	3+	1+
Canada field pea.....	—	1+	2+	3+	2+
Garden pea.....	—	2+	1+	2+	3+

The results presented above further substantiate the claims of previous investigators that the organisms of these four legumes, representing three genii of plants, will successfully cross-inoculate.

It is shown, however, that while we find that the organisms of this group cross-inoculate, in several instances the extent of nodulation, namely, the size and numbers of the nodules, is considerably less on the plant inoculated with a culture other than one of its own kind. This seems to be more marked in the case of the garden pea and Canada field pea plants than in that of the other legumes of this group. From these experiments, we would conclude that under ordinary conditions with the pea-vetch group, we could not expect as vigorous nodulation by using cross inoculation as by employing the organism of each plant directly.

*Cowpea group*

This group, according to Burrill and Hansen (1), entails not less than 9 different types of legumes. Of these, there are but 4 or 5 of any great importance in agriculture. These are the cowpea, Japan clover, velvet bean,

peanut and partridge pea. We have considered the first three of the above named in the work herein reported. These represent the genii *Vigna*, *Lespedeza* and *Mucuna*.

TABLE 4

*Showing the results of the cross-inoculation of B. radiculicola of the cowpea group*

KIND OF PLANT	RESULTS OF THE INOCULATION			
	Not inoculated	Cowpea	Japan clover	Velvet bean
Cowpea.....	—	3+	2+	3+
Japan clover.....	—	—(?)	1+	1+
Velvet bean.....	—	3+	3+	3+

Upon examining the above results we find that in all but one case inoculation was produced. The one in question, Japan clover plants inoculated with the cowpea organisms, failed to produce nodules each time the experiment was made. The tests were not as good as might have been possible if the Japan clover plants had not been so small. With this group of legumes, we again realize an irregularity in the extent of nodule production. It will be seen that the nodulation of the Japan clover plants in all cases was more or less poor.

#### DISCUSSION OF RESULTS

The results of the experiments with four of the principle groups of legumes corroborate the data of previous investigators, namely, that the organisms of plants of each group cause the formation of nodules on every other member of that group. With several groups there seems to be considerable variation in the extent to which the nodulation caused by cross-inoculation takes place. There is little doubt but that the organism isolated from the alfalfa plant and the one isolated from the roots of sweet clover are identical. Although the organism taken from the nodules of burr clover, belongs without doubt to the same group as the alfalfa-sweet clover organisms, nevertheless, the several different isolations of the alfalfa and sweet clover organisms always produced less vigorous nodulation on the roots of burr clover than the nodulation which they caused on their own original plants. This fact, taken into consideration with the differences in the nodulation in the pea-vetch and the cowpea group, brings to light the possibility that the organisms in cross-inoculation must adapt themselves to the conditions of their new host plant. This might be accomplished in a few generations of plants. Again one organism might be much less vigorous with regard to the manner in which it is able to attack the root of the legume, than is another organism of the same specific type. Hence, the results produced on plants of the same kind would be very different.

## SUMMARY

From the results of the experiments here reported, we summarize as follows:

1. *Bacillus radicicola* isolated from the roots of alfalfa, sweet clover and burr clover, all cross-inoculate. The alfalfa and sweet clover organisms cause but scant infection on the roots of burr clover.

2. The organisms isolated from any one of the 4 clovers, crimson, alsike, red, and white, caused as vigorous a nodule formation by cross-inoculation as upon its original host.

3. The organisms of the garden pea, vetch, Canada field pea and sweet pea cross-inoculated. By the cross-inoculation, the nodulation produced on Canada field pea and garden pea was not as vigorous as that resulting when the organisms isolated from each of these plants, respectively, were used.

4. With one exception, namely the Japan clover plant inoculated with the cowpea organism, the organisms of the cowpea group cross-inoculated.

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STATEMENT OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC.,  
REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

Of SOIL SCIENCE, published monthly at Baltimore, Maryland for October 1, 1918.

STATE OF NEW JERSEY }  
COUNTY OF MIDDLESEX } ss.

Before me, a Notary Public in and for the State and county aforesaid, personally appeared Jacob G. Lipman, who, having been duly sworn according to law, deposes and says that he is the Editor of SOIL SCIENCE and that the following is, to the best of his knowledge and belief a true statement of the ownership, management (and if a daily paper, the circulation), etc., of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, embodied in section 443, Postal Laws and Regulations, printed on the reverse of this form, to wit:

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JACOB G. LIPMAN, *Editor*.

Sworn to and subscribed before me this 21st day of September, 1918.

IRVING E. QUACKENBOSS, *Notary Public*.

My commission expires July 5, 1920.

[SEAL.]

# THE RELATION OF THE LIME REQUIREMENTS OF SOILS TO THEIR RETENTION OF AMMONIA<sup>1</sup>

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Several methods for determining the lime requirements of soils have been developed recently. New terms are being coined rapidly to designate various types of acidity which the methods propose to measure. A "lime requirement" determined by one method, in general may bear little relation to that secured by another.

The total amount of base removed by a soil from a solution of a neutral or alkaline salt is the resultant of at least two reactions proceeding simultaneously. These may be designated as (a) chemical fixation, and (b) physical absorption.

The number of basic ions removed from solutions of different bases will differ. In the first place, that removed for the neutralization of free acid is the same for various bases; but in many of our soils, the general idea is that the major portion of the base is retained by insoluble acid salts. Here an equilibrium is established. The extent to which the neutralization of these acid constituents proceeds before equilibrium is established depends upon (a) the activity of the base, or the extent to which it is ionized, (b) concentration, (c) temperature, and other factors.

What seems to be desired in a method for measuring "lime requirements" by the absorption of bases is that the neutralization of free acids shall be complete, and that the neutralization of the acid silicates shall proceed to about the same point for soils of quite different character before equilibrium is established.

It was this idea which prompted the work herein recorded. A study of various procedures has been made to see whether one could be developed which would give a "requirement" that would be, within reasonable limits, independent of (a) concentration of the base, (b) temperature, and (c) time of contact.

In adopting a base which would neutralize insoluble acid residues to about the same extent for soils of different reaction, the idea was held that to satisfy

<sup>1</sup> Contribution 227 from the Agricultural Experiment Station of the Rhode Island State College, at Kingston, R. I. Read at the 1916 Convention of the Association of Official Agricultural Chemists.

this condition there must be established, at the end of the reaction, circumstances in which no excess base should remain.

A volatile base, as ammonium hydroxide, suggested itself. The principle of the procedure was to treat the soil with ammonia water, evaporate to dryness, and measure the retained ammonia.

The procedure in detail as finally adopted, together with the effect of certain variations upon the absorption, are here recorded.

#### DISTILLATION BY AERATION

The aeration method seemed to possess desirable features for estimating small amounts of ammonia. However, as there is considerable discussion as to the virtues of this method, certain features had to be investigated. These were (a) selection of alkali, (b) aeration necessary, and (c) completeness of ammonia absorption.

*a. Selection of alkali.* Sodium carbonate, sodium hydroxide and magnesium oxide were used with the following results:

ALKALI USED	NH <sub>3</sub> RECOVERED			
	Absorption bottles		Blank soil	Corrected recovery
	No. 1	No. 2		
	mgm.	mgm.	mgm.	mgm.
Na <sub>2</sub> CO <sub>3</sub> (5 gm.) .....	51.34	-0.08	0.46	50.88
NaOH (2 gm.) .....	53.99	0.05	1.88	52.11
MgO (5 gm.) .....	48.68	-0.05		48.68
MgO (5 gm.) (distilling by boiling) .....	51.14		1.21	49.93

The use of NaOH permitted a somewhat more rapid recovery of ammonia, but the amount evolved was subject to greater variation than that recorded by the use of Na<sub>2</sub>CO<sub>3</sub>.

Distillation by boiling with MgO yielded results quite similar to those obtained by the use of sodium carbonate. The latter however, seemed to work very satisfactorily and was used as the alkali in connection with the results to be recorded.

*b. Aeration necessary.* With sodium carbonate as much ammonia was obtained by 18 hours aeration as by 60 hours, and the former period was adopted. Six determinations were conducted in a string and a current of 300 liters of air per hour [measured by the Kober method (1)] passed through the last flask.

*c. Completeness of ammonia absorption.* The figures recorded under (a) "Selection of alkali" indicate that the absorption is complete in the first bottle; that is, for amounts of ammonia up to 75 mgm., with the rate of aeration indicated.

These results indicate very clearly that the reaction which takes place between the soil and an aqueous solution of ammonia is rapid and is complete in one hour.

Small variations in temperature do not appreciably affect the amount of ammonia retained. However, at the lower temperature it is much more difficult to secure duplicate determinations. Evidently at these temperatures

TABLE 1

*Time of contact before evaporation, and its influence upon ammonia retained. (170 mgm. of ammonia added to 25 gm. of surface soil. Evaporated at 95-98° C.)*

RHODE ISLAND PLAT	½ HOUR	1 HOUR	4 HOURS	10 HOURS
No. 23: (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , unlimed*				
NH <sub>3</sub> retained (mgm.)		52.56	52.08	52.97
†Lime requirement (pounds CaO)		6,925	6,862	6,979
No. 25: (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , limed,*				
NH <sub>3</sub> retained (mgm.)		39.84	40.12	41.20
†Lime requirement (pounds CaO)		5,249	5,286	5,428
No. 27: NaNO <sub>3</sub> , unlimed*				
NH <sub>3</sub> retained (mgm.)		41.95	42.70	43.04
†Lime requirement (pounds CaO)		5,527	5,428	5,670
No. 29: NaNO <sub>3</sub> , limed				
NH <sub>3</sub> retained (mgm.)		28.35	29.44	28.49
*Lime requirement		3,735	3,879	3,753
No. 30: corn acre, rye plat‡				
NH <sub>3</sub> retained (mgm.)	18.56	19.38		
†Lime requirement (pounds CaO)	2,441	2,573		

\* Surface soil, 8 inches deep, collected June 29, 1916.

† Requirement of 2,000,000 pounds soil.

‡ Soil 12 inches deep, collected June 10, 1915, evaporated at 60° to 65° C.

TABLE 2

*Retention of ammonia dependent upon temperature of evaporation. [25 gms. soil, 170 mgm. ammonia (volume 50 cc.), 1 hour contact]*

	65-75° C.		95-98° C.	
	NH <sub>3</sub> retained	Lime requirement*	NH <sub>3</sub> retained	Lime requirement*
	mgm.	lbs.	mgm.	lbs.
Plat 23	52.56	6,925	52.22	6,880
Plat 25	40.66	5,357	39.98	5,267
Plat 27	43.38	5,715	43.79	5,769
Plat 29	30.39	4,007	28.08	3,699
Corn acre rye plat	19.92	2,629	17.68	2,329

\*Requirement of 2,000,000 pounds of soil in pounds CaO.

more ammonia is held by physical forces. Combinations with very weak organic acids might exist, which would decompose at the higher temperature.

In the smaller application of ammonia quite wide variation in retention results. In amounts of 170 to 340 mgm., the influence upon retention is practically negligible.



TABLE 3

*Retention dependent upon mass of ammonia added. (25 gm. soil, 1 hour contact, temperature 95-98° C.)*

	85 MGM. NH <sub>3</sub> ADDED		170 MGM. NH <sub>3</sub> ADDED		255 MGM. NH <sub>3</sub> ADDED		340 MGM. NH <sub>3</sub> ADDED	
	NH <sub>3</sub> re- tained	Lime require- ment*	NH <sub>3</sub> re- tained	Lime require- ment*	NH <sub>3</sub> re- tained	Lime require- ment*	NH <sub>3</sub> re- tained	Lime require- ment*
	mgm.	lbs.	mgm.	lbs.	mgm.	lbs.	mgm.	lbs.
Plat 23.....	45.49	5,993	51.74	6,817	53.04	6,988	53.38	7,033
Plat 25.....	32.91	4,336	39.16	5,159	40.52	5,338	40.18	5,294
Plat 27.....	33.45	4,407	43.65	5,751	43.04	5,670	42.77	5,635
Plat 29.....	22.37	2,947	26.77	3,527	27.47	3,619	28.49	3,753

	70 MGM. NH <sub>3</sub> ADDED		140 MGM. NH <sub>3</sub> ADDED		210 MGM. NH <sub>3</sub> ADDED		280 MGM. NH <sub>3</sub> ADDED	
	NH <sub>3</sub> re- tained	Lime require- ment†	NH <sub>3</sub> re- tained	Lime require- ment†	NH <sub>3</sub> re- tained	Lime require- ment†	NH <sub>3</sub> re- tained	Lime require- ment†
	mgm.	lbs.	mgm.	lbs.	mgm.	lbs.	mgm.	lbs.
Corn acre, rye plat†.....	19.38	2,573	20.74	2,732	23.12	3,046	23.12	3,046

\* Requirement of 2,000,000 pounds of soil in pound CaO.

† Temperature 60 to 65° C.

#### METHOD

Treat 25 gm. of soil in an evaporating dish with 50 cc. N/5 NH<sub>4</sub>OH. Stir the mixture occasionally during a period of one hour. Evaporate the solution to dryness on a water bath containing boiling water. Rub the soil up with a pestle and allow it to remain upon the bath for 1½ hours. Wash the soil into a 500 cc. Kjeldahl flask with 100 cc. of ammonia-free water. Add 5 to 10 gm. of sodium carbonate and distil by the aeration method [essentially the method used by Potter and Snyder (2)], keeping the flasks perpendicular and the bottom of the distillation tube slightly above the bottom of the flask. Absorb the liberated ammonia in 25 cc. N/5 H<sub>2</sub>SO<sub>4</sub> diluted to 200 cc. Determine the excess acid by titration with N/25 NH<sub>4</sub>OH, using alizarin red as indicator. Make blank determinations with each soil.

#### DISCUSSION OF METHOD

The procedure as outlined yields a requirement which is only slightly affected by wide variation in the conditions under which retention takes place. No other method tried by the writer begins to be so free from analytical weaknesses as the procedure outlined. In other soil types, however, it may fail absolutely to qualify even from a laboratory standpoint.

The real test, however, is what it will do in the way of indicating small variations in reaction, under field conditions.

No definite correlation with crop yields can be offered at this time. It had been hoped that the method may be submitted to a comparison with a crop sensitive to small variations in acidity, but such data cannot be presented now.

A few laboratory experiments have been made to learn to what extent the retention of ammonia can be used as an index of the absorption for other bases.

#### SUBSTITUTION OF BASES

The plan was to evaporate to dryness the soil treated with solutions of various alkalis and alkaline earths and record the effect upon ammonia retention. This was done in the case of sodium and potassium in the hydroxides, carbonates and chlorides, and with calcium and barium in the hydroxides.

The results indicated that the amount of ammonia retained was diminished in practically equivalent amounts when the soil was acted upon by the hydroxides and carbonates of sodium or potassium. It was diminished very slightly by the chlorides.

With barium and calcium hydroxide, the ammonia absorption was diminished only about one-half what it should have been when figured on the equivalent basis. Their reaction was much slower than that of the alkalis, and it was probably not complete. Some of the solution was probably carbonated and rendered inactive and would thus neither enter into combination with the soil nor by force of its alkalinity drive off its equivalent amount of ammonia.

In general, those bases which tend to cause deflocculation and dissolve organic matter are held in equivalent amounts as measured by the subsequent ammonia retention, while those bases which cause flocculation and become carbonated in the process of evaporation do not react in this way.

#### ABSORPTION OF AMMONIA BY CARBON BLACK

The well-known power of finely divided particles to hold gases upon their surfaces by physical forces led to the question of what the absorptive power of a substance like carbon black would be under the conditions existing in the method.

Consequently, 45 mgm. of ammonia in a volume of 50 cc. were evaporated from 5 gm. of carbon black. A blank determination of ammonia in the carbon black itself was made after evaporating with 50 cc. water.

	AMMONIA RECOVERED
	mgm.
Carbon Black, blank.....	6.59
Carbon Black + ammonia.....	5.84

It is seen that no ammonia is held under these conditions and it is believed therefore that little ammonia is retained physically by the soils under similar treatment.

*Comparison with the Veitch method*

	REQUIREMENT POUNDS CaO PER 2,000,000 POUNDS SOIL	
	Ammonia method	Veitch method
Plat 23 .....	6,925	8,714
Plat 25 .....	5,249	5,810
Plat 27 .....	5,527	8,070
Plat 29 .....	3,735	4,842

The requirement indicated by the ammonia method runs consistently lower, the greatest variation being in the case of soil from plat 27. The requirement indicated by the Veitch method for this soil is comparatively too high as measured by crop yields.

Soils treated according to the Veitch method yield an aqueous extract more alkaline than the extract of soils after evaporation with ammonia, which is nearly neutral.

ABSORPTION OF AMMONIA BY SOILS

After the completion of this paper there appeared in the literature a publication by Cook treating of the "Absorption of Ammonia by Soils" (3). The purpose of the present paper did not permit a review of the theories relative to ammonia absorption. The paper referred to makes such a contribution.

In the course of that paper it was pointed out that the application of calcium and sodium hydroxide resulted in an increased ammonia absorption in all cases but one. In the present paper it is shown that a decrease in retention of ammonia results from each of these treatments. It is desired merely to emphasize at this time that the method of procedure was entirely different, as given in two papers. Cook was working with solutions of ammonium sulfate at room temperature, while in the present work ammonium hydroxide was used and the excess eliminated at the temperature of boiling water.

The equilibrium that is established under Cook's treatment is referred to as being dependent upon numerous factors, among which are physical, physico-chemical, chemical and perhaps biological. The absorption which results from his procedure indicates that these factors are active.

In the present paper it is pointed out that the retention of ammonia which results from the equilibrium established by the procedure outlined is dependent upon fewer factors and seems to follow the direction of true chemical reaction.

Attention is called to Cook's work, and especially to the difference in procedure, lest a hasty review of his paper should give the idea that it is incompatible to use the "Retention of Ammonia" as an index of lime requirements.

## SUMMARY AND CONCLUSIONS

A procedure is outlined for determining the lime requirements of soils, which consists in treating the soils with ammonium hydroxide, evaporating off the excess ammonia at a temperature of boiling water, and estimating the retained ammonia.

Within reasonable limits the requirement based upon this retention was independent of (a) concentration of ammonia added, (b) time of contact, and (c) temperature during evaporation.

The requirement was about 25 per cent lower than that indicated by the Veitch method.

Aeration in the presence of sodium carbonate for 18 hours (300 liters of air per hour) is sufficient to remove completely 50 to 75 mgm. of ammonia from the soil.

Sodium, ammonium and potassium from solutions of their hydroxides and carbonates are retained in practically equivalent amounts.

It is believed that the ammonia retained is held chemically by a neutralization of either free acids, acid organic compounds or acid salts, while physical absorption is largely prevented.

The "requirement" based upon the ammonia retention agreed in general with field observations, to the extent that soils needing the most lime showed the greatest "requirement."

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# THE EFFECT OF ORGANIC MATTER ON SOIL REACTION

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## INTRODUCTION<sup>1</sup>

Organic material, which is largely protein in nature, is very complex in composition, and its destruction results in many complicated reactions, both synthetic and analytic. The external factors which effect these reactions are subject to continuous fluctuation and hence equilibrium probably never exists. It would seem quite reasonable to assume that in general the reactions follow certain general tendencies. For example there may be a periodic production of acidity or of basicity; or of both, depending upon the stage of decomposition. This work has been planned to study the effect of organic matter on the production of acids or bases in the soil.

Very little work along this line has been reported. White (20,21) of Pennsylvania carried out pot experiments on the effect of poultry and stable manure and fresh and dried green manures upon soil acidity (Veitch Method), using the common farm crops and sorrel as green manures. A considerable reduction in limestone requirement was shown during the first two weeks, but this reduction might have resulted from the mere addition of the organic materials and not been due to decomposition processes at all. Red clover was found to be the best corrective of acidity, while wheat and corn increased the lime requirement more than any of the other materials. Changes in reaction during the second and third months seem to have been very slight. In general the effects produced were quite varied and fluctuating.

Skinner and Beattie (17) studied the effect of certain mineral fertilizers as well as organic materials, upon soil reaction. They found that sulfates increased the acidity, while organic matter had a varying effect. Starch increased the acidity. Manure caused only a slight increase, and leachings from manure caused less acidity than was present in untreated soil. They conclude that the nature of the decomposition of the organic material in the soil and the character of the life processes in the soil affects the influence of such substances on soil reaction.

Miller of Missouri (13) reports that manure, wheat, straw, green oats, dry oats, and clover in the soil caused a decrease in soil acidity for the first 8

<sup>1</sup> Acknowledgments: This work was planned in consultation with Dr. P. E. Brown and Dr. R. S. Potter to whom credit is due for many helpful suggestions and criticisms.

weeks, after which there was a gradual increase until at the end of 26 weeks, the treated soils were as acid as the untreated. The experiment indicates that ordinary green crops and manure turned under do not increase acidity, but that the use of a sugar-containing crop, such as sorghum, may have this effect for a few weeks.

These reports give the only available results bearing directly upon the subject, and in general they indicate that the effect upon soil reaction depends very much upon the nature of the organic matter used.

#### THE COMPOSITION OF ORGANIC MATTER

##### *Nitrogenous material*

There are two kinds of organic material to be considered here, nitrogenous and carbohydrates. The nitrogenous materials consist principally of proteins or their degradation products. A small amount of nitrogen may of course be in the form of nitrate or ammonium salts, some in the form of nitrogen bases, including alkaloids, which are the basic substances of plants; ptomaines, basic substances of animal origin, and other products representing the various stages of protein decomposition. Purine and pyrimidine bases some of which have been isolated from soils, may arise from the breaking down of nucleic acids; and choline, also a strong base, is a product of lecithin disintegration. These are the principal sources of nitrogen other than proteins.

The breaking down of protein materials is a hydrolytic process. The first nitrogen liberated is probably that from the amide groups. Then further decomposition leads to breaking of the amide linkages, and the production of free amino acids. These acids are an easily available source of energy for many bacteria and molds and are further decomposed into bases and acids. The particular kind of decomposition depends upon aeration and other factors. There may be either a decarboxylation or deamination or both processes may occur. In general anaerobic bacteria reduce alpha-amino acids forming saturated fatty acids and ammonia. Aerobic bacteria tend to oxidize alpha-amino acids to a fatty acid containing one less carbon atom, producing at the same time carbon dioxide and free ammonia. Four type reactions which illustrate the different decomposition processes most likely to occur are given below as taken from Dakin (4).

- |                                   |  |
|-----------------------------------|--|
| 1. $RCH_2 - CHNH_2 - COOH + H_2$  | $R - CH_2CH_2COOH + NH_3$ (Reduction)    |
| 2. $RCH_2 - CHNH_2 - COOH + O_2$  | $R - CH_2COOH + NH_3 + CO_2$ (Oxidation) |
| 3. $RCH_2 - CHNH_2 - COOH + H_2O$ | $R - CH_2OH + NH_3 + CO_2$ (Hydrolysis)  |
| 4. $RCH_2 - CHNH_2 - COOH$        | $RCH_2CH_2NH_2 + CO_2$ (Decarboxylation) |

Other kinds of reaction might occur, and these acids, amines and alcohols are of course, further decomposed. Such reactions though not studied originally from the soils standpoint show very well the general nature of processes likely to be occurring in the soil.

As the decomposition of protein proceeds, therefore, it is entirely possible for acidity to increase quite markedly. The glycine and alanine that is split off may produce acetic and propionic acids, serine may yield propionic and formic acids; valine and leucine, isovalerianic, and isovaleric acids; and other amino acids may likewise yield an organic acid. In this study a strong odor of butyric acid was evident in the first stages of decomposition. At the same time basic compounds may be produced and these nitrogen bases may likewise be ammonified. Ammonia will, of course neutralize acids, as will also other nitrogen bases. These, in the absence of mineral bases would tend to permit nitrification, but the production of nitric acid would again make further demands for base. At the same time, so far as results show, the organic acids which might have accumulated would probably gradually oxidize and disappear. It would even be possible that organic acidity might never accumulate under any condition of adequate aeration. But since soils are so variable in character and since widely different systems of management are employed, no definite conclusions may be drawn.

#### CARBOHYDRATE MATERIALS

The mixture of degradation products of soil organic matter, which is too loosely classed as humus is perhaps somewhat influenced also in its reaction by carbohydrate materials. Though such materials may produce acids as acetic, propionic, and butyric, it is not known for how long a time they may exist unchanged. Natural albumin acids seem to disintegrate more slowly than the carbohydrate acids. Snyder of Minnesota (18) found that manure and other nitrogenous materials produced a humus of greater acidity than did straw and sawdust which contain less nitrogen. The fact that residues of a cellulose or starchy nature require more time for decomposition, makes possible a more complete oxidation of any acidity which might accompany the decomposition. In general, it seems that the most available carbon disappears from the soil rather more rapidly than does the nitrogen, so that, at times, it becomes a matter of importance to apply carbon-rich manures. The relative values of the different types of materials under the different systems of soil treatment is, however, a subject for further investigation.

#### PRESENTATION OF THE PROBLEM

It has been observed that very complex reactions are occurring continuously in the soil. These may be tending simultaneously toward acidity and basicity in soil reaction. Chemical potential demands that reactions shall occur in such a way as to result in the maximum entropy to a given system. As carbon dioxide is evolved from the soil or nitrogen is lost or used by plants or a hundred and one other reactions occur, there is a change in the chemical potential. Aside from the purely chemical, there are also what may be termed



life forces, including the action of any and all soil life. Even the enzymes of origin external to the soil may have an effect. For example glucosides contained in plant cells, are broken down by enzymes which are present in other cells from which they must be liberated before attacking the glucoside. The freeing of these enzymes and their activity upon plant residues may hasten availability of nutrients, both saccharine and non-saccharine in nature, to soil organisms. Physical factors in the soil may favor or disfavor certain of these ends and at times results appear in reverse order to expectations. Arbitrary statements should not be made, therefore, in regard to data from a study, which is at best, conducted under more or less unnatural conditions and burdened with many limitations.

It may be presumed that no material ever becomes of use as a fertilizer until some organism breaks it down into a simpler form. The changes are brought about by an immense number of organisms, the end products depending upon the nature of the substrate and the environment. Usually the more desirable products are favored by aerobic activity, but the conditions cannot always be regulated in either field or laboratory. It is probable, however, that for every molecule of ammonia split off there may be formed at the same time under any condition an equivalent of amount of acid, even though the molecule may exist only momentarily.

Whatever deleterious effects may be produced by such transitory acids are probably not worthy of consideration. When organic matter is added to soils there is not only an increased absolute amount of food, but reactions which occur lead to an increased variety of degradation compounds. Since every variety of substance, be it acid or base, is select food for a special organism or group of organisms, a more multitudinous and varied flora follows the addition of organic materials. Greater bacterial activity means increased available mineral food, due to the dissolving action of acids or carbon dioxide produced. This effect should be perceptible in the rate at which calcium carbonate added to the soil is decomposed. Consequently in these studies residual carbonates, as well as acidities, have been determined. The disappearance of carbonate and increase in acidity may not necessarily run parallel. In fact later data show that carbonates have practically all disappeared on treatments which have diminished the acidity, while there is yet a portion of the carbonate remaining on treatments which have increased the lime requirement. By combining acidity, carbonate and nitrogen changes a more intelligent interpretation of the various soil reactions may be reached.

#### PLAN OF EXPERIMENTS

Typical of highly nitrogenous materials, albumin, casein and blood have been studied. Starch and dextrose have been used as carbohydrates. And as materials of more practical significance alfalfa and ammonium sulfate have been employed. Two soils were tested with all treatments. The studies

were made in the greenhouse using gallon earthenware jars for containing vessels. Four samplings were made at intervals of 2, 5, 10 and 15 weeks, respectively, thereby covering a total period of a little more than 100 days. The results by White (20, 21) were reported for several months, a much longer period, as were those by Miller (13). Skinner and Beattie (17) made field studies covering a period of 5 years. In this study, however, rather easily decomposable materials were used and it is doubtful if more significant results might have been obtained from a longer study. Ordinarily crop residues might require a longer time for decomposition.

On each sampling 4 determinations were made, the ammonia, nitrates, acidity and carbonates of limed soils. On the second and last samplings Dr. Potter had planned to determine the soluble non-protein and perhaps other forms of nitrogen, but circumstances made it necessary to report the results incomplete as will be seen in the following paper by Potter and Snyder.

The soils were quite distinct in type. One was from the humus plots of the station, a dark soil fairly rich in organic matter, classified as Carrington silt loam. The other soil was from the Agronomy farm, rather sandy, light in color, and low in organic matter, probably classified as Carrington sandy loam.

Most of the organic treatments were made at the rate of 10 tons per acre. One application on the humus soil was made at double this rate for alfalfa and the ammonium sulphate was added in all cases at the rate of only one ton per acre.<sup>2</sup> It might be argued that these are excessive treatments, and that they would not apply to practical farm conditions. But when it is remembered that there is a more thorough mixing and that the materials not naturally existing in that condition are more finely ground than in field treatments, it would not seem likely that any physical effect produced by the treatment here would be greater than that occurring naturally in soils. Organic matter turned under in the field is likely to occur in a layer which is not well distributed and the concentration of material locally might be even greater than that prevailing in the pot treatments. It would not be maintained, however, that identical decompositions would occur.

Optimum moisture conditions were provided as nearly as possible by daily watering. Though pots cannot be controlled as accurately as tumblers, and checks are consequently not as good, the results are more nearly comparable with field conditions and of more value for that reason. Moisture and temperature fluctuations are perhaps greater in the field. These studies began in April when greenhouse temperatures were relatively low. In July and August on the other hand, extremes of 115° to 120°C. were reached. Moisture conditions were subject to similar changes. In very hot dry weather the surface soil of the pots became quite dry even with daily watering. In moist weather on the other hand, the soils never dried out and demanded only a little water

<sup>2</sup> All calculations are made on the air dry basis.

once or twice per week. These variable conditions, no doubt, had their effect upon the results obtained, but they may be regarded as indicative of what may occur in the field. There is not, however, the leaching and over saturation of water, or the effect of plant growth to which field treatments would be subject.

#### AMMONIFICATION<sup>3</sup>

The ammonia produced was determined by the aeration method using potassium carbonate to free the ammonia from its salts. The method has proved very accurate, as duplicates agree closely, even when corresponding determinations are run at different times. After the first sampling it was decided to run only one determination since differences due to the various treatments were usually quite marked and variations due to manipulation of the method were not significant. Though ammonification probably began in a few hours or at most in a few days, information on this phase of the question was not especially desired and determinations were not made except at the regular periods of samplings. The results are shown in table 1.

With albumin and casein it is observed that the greatest amount of ammonia was found at the first sampling on both soils. The chief cause for the large amounts of ammonia at first is the failure of nitrification to begin. It may be noted that there are no nitrates at the first sampling except in the casein-treated pots of the humus soil. The multiplication of organisms, however, may be inferred to have been occurring at this time. Consequently more and more of the changed materials is being resynthesized as the experiment progresses. It is notable too, that though casein seems to have nitrified more quickly than albumin, there is also a greater accumulation of ammonia, showing more rapid progress of both ammonia and nitrate production at first. After the first sampling the results are fluctuating. By far the largest absolute amount of ammonia is found in the humus soil, indicating a superior ammonifying power for this soil. With blood on the other hand the largest amount of ammonia is found at the last sampling in spite of the fact that there is greater nitrate production at the same time. This is illustrative in a quite definite way of the difference in ease with which different organic materials are decomposed.

The carbohydrate materials give such small amounts of ammonia that interpretation of the results is not significant. Since no nitrogen is added by starch or dextrose no great amount of ammonia could be expected. As a matter of fact the untreated soil produced practically the same quantity. Alfalfa and ammonium sulfate behave in much the same way. A little more nitrogen is added in the alfalfa but it is probably not quite as rapidly available. After the first sampling there is no more ammonia with alfalfa treated soil, than

<sup>3</sup> All results with ammonification and nitrification are expressed as parts of nitrogen per million parts of soil.

TABLE 1  
*Amount of ammonia at the end of each period*

TREATMENT	1ST SAMPLE, 2 WEEKS		2ND SAMPLE, 2 WEEKS		3RD SAMPLE, 10 WEEKS		4TH SAMPLE, 15 WEEKS	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
Humus soil	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
Soil alone..... { 1	14	16.8	5.6	11.2	16.8	11.2	16.8	11.2
2	16.8	21.0	11.2	22.4	5.6	16.8	16.8	16.8
Albumin..... { 1	982.8	676.4	588.0	173.6	632.8	100.8	856.8	112.0
2	917.0	705.2	666.4	168.0	800.8	72.8	588.0	78.4
Casein..... { 1	917.0	855.3	515.2	448.0	582.4	56.0	627.0	39.2
2	982.8	784.0	582.4	448.0	532.0	33.0	448.0	28.0
Starch..... { 1	22.4	14.0	11.2	16.8	16.8	11.2	16.8	11.2
2	21.0	7.0	16.8	16.8	16.8	16.8	16.8	11.2
Blood..... { 1	350.0	299.6	352.8	448.0	526.4	140.0	548.8	100.8
2	393.4	322.0	386.4	392.0	487.2	28.0	560.0	22.4
Dextrose..... { 1	23.8	23.8	16.8	11.2	16.8	16.8	28.0	11.2
2	23.8	14.0	16.8	16.8	28.0	16.8	28.0	11.2
Alfalfa..... { 1	22.4	19.6	5.6	16.8	16.8	16.8	16.8	11.2
2	19.6	16.8	16.8	16.8	5.6	22.4	11.2	11.2
Double alfalfa. { 1	39.2	19.6	16.8	16.8	16.8	11.2	11.2	11.2
2	30.8	22.4	16.8	16.8	16.8	16.8	16.8	16.8
Ammonium sulphate.... { 1	95.2	22.4	50.4	16.8	28.0	22.4	16.8	11.2
2	98.0	14.0	50.4	5.6	22.4	16.8	16.8	11.2
Sandy soil								
Soil alone..... { 1	11.2	11.2	11.2	11.2	11.2	16.8	16.8	11.2
2	11.2	16.8	11.2	16.8	11.2	22.4	11.2	22.4
Albumin..... { 1	660.8	546.0	616.0	324.2	571.2	509.6	560.0	313.6
2	672.0	504.0	621.6	358.4	711.2	476.0	521.2	268.8
Casein..... { 1	809.0	761.6	532.0	156.8	543.0	229.6	672.0	117.6
2	786.8	772.3	588.0	285.6	616.0	207.2	599.2	44.8
Starch..... { 1	22.4	16.8	11.2	11.2	11.2	11.2	16.8	22.4
2	11.2	5.6	11.2	11.2	11.2	11.2	22.4	16.8
Blood..... { 1	498.4	408.8	420.0	448.0	481.6	151.2	565.6	67.2
2	518.0	408.8	408.8	392.0	481.6	212.8	565.6	140.0
Dextrose..... { 1	11.2	11.2	11.2	11.2	16.8	16.8	22.4	5.6
2	16.8	11.2	11.2	11.2	22.4	11.2	44.8	11.2
Alfalfa..... { 1	39.2	33.6	11.2	11.2	11.2	5.6	11.2	11.2
2	39.2	16.8	11.2	11.2	11.2	22.4	11.2	11.2
Ammonium sulfate..... { 1	134.4	72.8	95.2	11.2	123.2	11.2	78.4	28.0
2	140.0	89.6	95.2	5.6	112.0	16.8	72.8	16.8

with untreated, due to nitrification. The ammonium sulfate pots run a little higher, especially on the untreated sandy soil. This is due to lack of nitrification in the absence of lime, since the ammonia obtained in the determination represents the unchanged fraction of the ammonium sulfate. On the humus soil which gave a higher lime requirement, however, active nitrification occurred.

The effect of the lime in depressing ammonification is very characteristic, agreeing with results obtained by previous workers, where rather large amounts of lime were applied. (Six and seven tons, respectively, on the sandy and humus soils were used). Where the nitrogenous organic materials are added the depression is quite extreme. Part of the depression is only apparent, however, since nitrification is much greater in the presence of lime. But the summary table shows that there is yet a depression in nearly every case except where ammonium sulfate is used even when allowing for the change to the nitrate form. Still another factor may enter here. Organisms are usually more numerous in the presence of lime if soils are acid, and a greater number of organisms might have resulted in a greater amount of both nitrate and ammonia being synthesized into new proteins. The importance of this factor is questionable.

The nitrogen content of the materials used was 15.24 per cent, 14.25 per cent, 14.48 per cent, and 2.92 per cent, respectively for the blood, casein, albumin, and alfalfa. Since this is adding nitrogen at the rate of 1524, 1425, 1448, and 292 parts per million of soil, it is easy to calculate the per cent which is changed to ammonia at any time. The casein and albumin approximate 50 to 60 per cent at the first sampling on the humus soil and 40 to 50 per cent on the sandy soil at the same time. Blood starts at about 20 per cent, and runs up to something like 30 per cent, on the humus soil but starts at better than 30 per cent, on the sandy soil and never runs much higher. Only a small per cent of the nitrogen of the alfalfa even exists in the ammonia form.

The general behavior of the two soils is noticeable because of characteristic differences. The sandy soil promotes a slower ammonification in practically every case except for blood. The effect of lime in depressing ammonification is apparently greater on the humus soil at the last two samplings. Previous to that time there is less difference.

The cause for greater ammonification in the absence of lime, aside from the differences due to change to nitrate, may be due to differences in the soil flora. In acid soils a large part of the ammonification may be due to fungi. This is in accordance with the conception that organic soils contain chiefly molds, rather than a bacterial flora. Though these soils are not especially humus-rich, a rather heavy application of organic material has been made, and the acidity by depressing bacteria, would tend to stimulate mold growth. Though molds may be abundant even under conditions which furnish an alkaline reaction, bacteria do not thrive in acidity, and there is, therefore, less competition to the mold organisms. Contributory to this thought McLean and Wilson

(14) of New Jersey found that moulds were very efficient ammonifiers, converting nearly half the nitrogen of dried blood in 7 days. It is known, also, that ammonification occurs in flooded soils on which rice is grown where mold growth and anaerobic bacteria would be responsible for the change.

A preliminary test with tumblers has shown that molds are very abundant in these soils made artificially acid with sulfuric acid and treated with 10 tons of albumin. The greater the acidity the greater the mold growth has been up to a certain limit at least. The normal soil showed little mold growth, and in fact, there was no great amount of visible mycelium or fruiting bodies until an acidity equivalent to 7.2 tons including that already in the soil was supplied. The 8.5 and 9.6 ton acidities developed likewise a heavy growth. Later just as heavy a growth appeared on the 17.5 ton requirement. Soils made acid without the addition of organic matter did not develop a heavy visible growth, showing that a combination of the two factors, acidity and

TABLE 2

*A test in tumblers treated with  $H_2SO_4$  after 5 weeks incubation, humus soil only*

	ACIDITY	NITROGEN AS AMMONIA	NITROGEN AS NITRATES	SUM OF NITRATES AND AMMONIA
	<i>tons</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
Soil, albumin.....	3.7	700.0	255.1	955.1
Limed soil, albumin.....	2.7	453.2	416.6	869.8
Soil, albumin, acid.....	4.3	761.6	184.5	946.1
Soil, albumin, acid.....	6.1	770.0	143.7	913.7
Soil, albumin, acid.....	7.2	820.4	71.2	891.6
Soil, albumin, acid.....	8.5	795.2	53.4	848.6
Soil, albumin, acid.....	9.6	828.8	32.4	861.2
Soil, albumin, acid.....	17.5	728.0	Trace	728.0

organic matter was most favorable to mold production. In every case high acidity has favored molds as would be expected. The object in making the heavy application of acid was to intensify differences. These conditions make it possible to determine in a limited way the activity of the mold flora of the soil, and the results should suggest the capacity of molds for taking part in the nitrogen changes even under normal conditions. Of course, acid resistant bacteria might also be of significance. It is possible that the acid may denature the albumin by forming acid albumin yet the effect in this respect may not be vastly different from that which occurs in soils without the artificial acidity. More definite results from this study are reported below.

The addition of the albumin shows the same depressing effect upon the lime requirement of the normal soil. Sulfuric acid was chosen to increase the acidity because it is a strong mineral acid, and the sulfate ion would probably not have a deleterious effect on the organisms. There were duplicate tumblers but the checks agreed so well that only averages are presented in the table.

There is the same depression of ammonia and increase of nitrates in the presence of lime (the treatments were the same as in the original experiment except for the  $\text{H}_2\text{SO}_4$ ). The absolute amount of ammonia is found to increase with increasing acidity fairly regularly up to the heaviest acid treatment. Even here there is only a slight depression. On the other hand the nitrates decrease quite regularly with increasing acidity until there are none or only a slight trace in the most acid treatment. Taking the sum of nitrate and ammonia nitrogen, the maximum is found in the normally acid soil and the next highest amount at the next acidity and on down until there is a lime requirement of 8.5 tons before there is a depression. The indication is, therefore, that ammonification continues under rather extreme acid conditions, while nitrification is much depressed and finally stopped by the same conditions.

Correlating these results with the very heavy gray and yellow mold grown in the tumblers it would seem quite probable that the ammonification was due to considerable extent to the molds. Yellow fruiting bodies were very abundant and the moldy odor was quite conspicuous. In the pot treatments, also, a rather conspicuous growth of a lead gray mold was observed. The evidence is at least in favor of the activity of molds as accounting for the greater ammonification under acid conditions rather than in the presence of lime.

A weak point in the study is of course the incapacity of the method used for determining hydrogen-ion concentration. But since sulfuric acid is a strong acid and enough was present actively so that an acid extract was obtained from the treated soils, it may be presumed that a higher concentration of acidity prevailed than is present in normally acid soils. The water extract showed increased acidity with increase in acid applied so far as the color of methyl orange could indicate.

Some discussion as to the significance of ammonia and other forms of nitrogen aside from the nitrate, in the nutrition of plants may be of indirect relation to this study. New conceptions are formed as the nitrogen problem is better understood. It has been suggested (10) that since the plant must reduce nitrate to ammonia in order to synthesize protein a saving of energy would result if a more complex form of nitrogen than ammonia such as an amino acid were used by the plant. Complying with this idea investigators have made tests, and a number of compounds, including arginine, histidine, xanthine, hypoxanthine, guanine, creatine, choline and nucleic acids, have been found to be directly beneficial to plants. Some of the materials have given results indicating that they were a better source of nitrogen than is nitrate itself. Rice which grows in the absence of nitrate uses ammonia and does not thrive in the presence of nitrate.

Along the same line Lipman (12) suggests the possibility of a diffusable compound passing from the roots of legumes and stimulating the growth of a non-legume. Such a postulation seems entirely reasonable in the light of the knowledge that such compounds as urea, glyocoll, asparagine, and lucine,

serve as sources of nitrogen for plant growth. It would not, therefore, be necessary that these compounds which are soluble and diffusible be nitrified before they could be of use to the non-legume. These same substances are also products of both analysis and synthesis by organisms, which not only makes possible beneficial effects of many organic manures as a source of nitrogen before nitrification occurs, but makes it likely that some benefits may accrue even in the absence of an efficient nitrifying flora. Compounds of greater complexity than dipeptids are relatively insoluble and beneficial effects due to their presence could hardly be expected. There is a slight possibility also that the beneficial effects sometimes attributed to micorhizal growths may be due to partially synthesized nitrogen compounds of no greater complexity than dipeptids, which the host plant is able to absorb from the micorhizal filaments. According to Coville (3) the fungi themselves habitualy use organic nitrogen which they may obtain as well from acid soils. If then, as he also suggests they supply the host plant with some sort of nitrogen they become of double value, by supplying the nitrogen and likewise by saving energy to the host plant.

In the future it would seem at least justifiable to refrain from the declaration that plants must have nitrates. The solubility and nontoxicity of nitrate and the reaction products makes it an especially suitable plant food. Yet it is observed that when plants, as in the case of rice, fail to develop the necessary reducing enzyme to make use of nitrate in protein synthesis, there is no loss in thrift or efficiency of the plant organism.

And again when it is observed that although molds may not nitrify, they do produce ammonia, amino acids and dipeptides which are probably of direct use to plants, their activity has greater significance. The fact that more productive soils usually permit greater nitrification may be more incidental than fundamental. A well aerated soil is essential to productiveness but it likewise stimulates the production of nitrates. Under such conditions protein degradation products other than nitrates perhaps have little opportunity to function in the nutrition of plants. And while it is a fact that plants use nitrates it is also a pertinent question as Lathop (10) has suggested, to ask why it should be necessary.

It is not desired, however, to minimize the importance of the nitrifying process in soils. It is only by those oxidation processes that toxic substances are prevented from accumulating and such oxidations insure, thereby, the recovery of both carbon and nitrogen to serve again a cycle of usefulness.

#### NITRIFICATION

For the determination of nitrates the phenol-disulfonic acid method, as modified by Davis (5), in this laboratory, was used. The method has the advantage of accuracy and ease of adjustment to the quantity of nitrates by the making of proper dilutions. There is also the advantage of detecting small amounts of nitrate. The results are shown in table 3.



TABLE 3  
Nitrates at each successive sampling

	2 WEEKS		5 WEEKS		10 WEEKS		15 WEEKS	
	No lime	Lime	No lime	Lime	No lime	Lime	No lime	Lime
Humus soil	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
Soil alone.....	17.3	26.3	40.2	68.6	55.9	126.6	43.3	175.8
	17.7	26.3	40.4	93.7	62.4	187.3	87.6	215.6
Albumin.....	Trace	Trace	159.0	150.0	189.6	413.1	448.4	936.6
	Trace	Trace	223.2	236.2	275.6	589.5	451.3	664.6
Casein.....	67.0	26.0	395.5	620.9	524.4	789.6	468.7	1530.0
	80.6	26.0	468.7	427.8	414.3	774.6	684.7	1360.5
Starch.....	0.0	00.0	0.0	0.0	Trace	Trace	35.8	124.2
	0.0	00.0	0.0	0.0	70.5	77.1	89.4	185.4
Blood.....	83.4	157.0	375.5	485.7	291.6	429.5	492.2	542.8
	82.2	165.2	287.6	564.8	443.5	559.7	327.7	1345.0
Dextrose.....	Trace	Trace	23.5	34.5	53.2	73.6	91.1	133.2
	Trace	Trace	14.3	41.3	48.3	83.2	71.7	124.9
Alfalfa.....	58.4	65.7	138.4	135.5	157.8	150.1	318.0	259.8
	45.7	55.2	118.7	154.2	183.3	177.6	337.9	269.8
Double alfalfa.....	83.1	87.7	188.6	150.1	255.7	421.2	513.5	515.0
	58.7	80.4	179.5	159.2	300.3	310.2	180.6	324.1
Ammonium sulfate.....	40.7	123.2	109.5	197.8	95.5	317.4	138.7	302.0
	52.8	119.3	127.7	202.0	155.5	255.2	176.8	298.6
Sandy soil								
Soil alone.....	10.9	19.0	9.1	17.4	28.4	49.3	38.3	51.4
	10.9	19.0	10.4	16.4	28.4	47.7	36.7	41.8
Albumin.....	Trace	Trace	13.4	52.0	184.5	103.4	342.0	512.5
	Trace	Trace	14.0	13.0	97.5	118.5	241.4	558.6
Casein.....	Trace	Trace	30.0	42.0	149.8	353.9	408.1	645.3
	Trace	Trace	23.0	30.0	178.1	599.5	264.4	430.6
Starch.....	Trace	Trace	Trace	Trace	Trace	Trace	Trace	29.6
	Trace	Trace	Trace	Trace	Trace	Trace	Trace	44.1
Blood.....	31.6	41.4	351.9	425.1	314.6	560.9	676.9	717.6
	36.8	36.3	279.0	425.0	231.8	424.9	358.8	565.7
Dextrose.....	Trace	Trace	Trace	Trace	Trace	Trace	22.9	52.5
	Trace	Trace	Trace	Trace	Trace	Trace	26.1	46.5
Alfalfa.....	17.5	22.0	70.4	83.8	87.4	114.7	196.1	246.8
	26.9	21.4	72.1	96.2	112.5	118.9	202.1	176.8
Ammonium sulfate.....	22.6	56.9	58.5	192.5	74.2	128.9	206.1	374.0
	22.7	45.3	63.1	167.4	89.5	179.3	133.6	370.0

It is noticeable that the highly nitrogenous materials, albumin on the humus soil and casein likewise on the sandy soil, failed to show nitrates at the first sampling. This is probably due to a slight toxicity caused by the accumulation of ammonia at first. Then the rapid multiplication of organisms resulted in the exhaustion of the original soil nitrates before active nitrification had time to begin. Nitrification in practically all cases is found to increase to the last sampling. Casein nitrifies most rapidly of any of the nitrogenous materials, on the humus soil, but on the other soil blood takes the lead. Blood also begins nitrification at once on both soils apparently because ammonification did not begin at such a rate as to render its accumulation toxic. Consistent with its character in ammonification, blood nitrifies gradually, and finally produces the maximum absolute amount of nitrate in the sandy soil. In the humus soil, casein holds this place, though one pot in the last sampling of blood was a close second. With the exception of this one determination, blood is second to albumin on the humus soil. In contrast to the rapid nitrification of blood, albumin fails to nitrify at a comparable rate until the last sampling on the sandy soil, and not until the third sampling on the humus soil. Casein on the other hand catches up with the blood at the second sampling on the humus soil, and somewhat at the third on the sandy soil.

Starch and cellulose behave in much the same way on both soils since nitrates disappear almost entirely and continue absent nearly to the end of the experiment. There are two explanations for this action. Either denitrification may occur in the presence of easily available sources of energy, or nitrification never sets in for the same reason. It is probable that the organisms do not nitrify except to the extent of satisfying the nitrogen demands of the soil flora. Such results would suggest a means for control of the rate of nitrification. In fact Kelly (9) has reported results showing a reduction of 50 per cent or more in the rate of ammonification of casein and blood, in the presence of starch. Brown and Kellog (1) obtained a similar depression of the oxidizing power of organisms in sulfonation studies. They found that cane sugar, starch, and filter paper all depressed the oxidation of the sulfur, the greater the amount and the solubility, the greater the depression. Gerlach and Densch (8) found that easily decomposable organic compounds such as dextrose or straw caused soluble nitrogen salts such as ammonium sulfate and sodium nitrate to be converted into protein compounds in the soil. Later, however, these were readily decomposed. Similar results were obtained also in work done at New Jersey. Lipman, et al. (11) has suggested the possibility that the large evolution of carbon dioxide in the presence of carbohydrate material might account for the non-appearance of nitrates. Coleman (2), however, found that a smaller amount of dextrose (0.5 per cent) increased the rate of nitrification. This increase may be attributed to increased numbers of organisms which soon exhaust the dextrose and then attack the nitrogenous organic matter quite vigorously. Brown suggests a temporary

toxicity to the nitrifying flora as a possible cause of non-appearance of nitrates. The results are illustrative of the inter-relationships of carbohydrate and protein organic portions of the soil, and suggest a problem for study to determine more definitely what the relationship may be. It might be possible to exercise a measure of control over nitrogen changes by making proper protein and carbohydrate combinations.

Wright (22) in making a study of various organic materials upon nitrification concluded that coarse material plowed under in the undecayed state, may materially reduce the quantity of available nitrogen in the soil. Plowing under such materials in the fall should, therefore, save nitrogen from loss during the winter and by spring permit nitrification sufficient for crop production. This author found, however, that green and succulent material was rather easily and quickly attacked. Evidently there are several factors which may determine the susceptibility to nitrification and, therefore, the more advisable time and rate of application of an organic fertilizer.

In line with the results it may be inferred that nitrification in excess of nutritional demands begins when easily available carbohydrate is exhausted. Nitrification starts in every case to an appreciable extent, except on the unlimed starch-treated sandy soil. Nitrification begins soonest on both soils in the presence of dextrose, probably because dextrose is more easily available and is, therefore, more quickly exhausted. The insolubility of the starch and the protective covering of cellulose about the starch would render it quite resistant, and probably result in a large residue of unavailable material. The limed treatments start nitrification first and produce greater quantities of nitrates, which is also consistent, since more organisms should be active in the presence of lime, and carbohydrate would, therefore, disappear more rapidly. The argument applies again consistently in that in the humus soil which should possess a more multitudinous flora to exhaust the carbohydrate, nitrification begins first in the presence of both starch and sugar. Evidence, therefore, points to lack of nitrification rather than to denitrification as the cause of absence of nitrates.

Doryland (6) presents results which are in line with the above conclusions and in his publication may be found also an excellent historical review and bibliography. The results of previous workers in general, justify the interpretation presented in this paper.

Alfalfa behaves in much the same way as blood, gradually increasing in absolute amount of nitrates up to the last sampling. It is notable, however, that there is not twice as much nitrate produced when the rate of adding alfalfa to the soil is doubled. In only a few cases is it nearly double. Ammonium sulfate makes very consistent and gradual increases up to the last sampling.

The difference in the behavior of the two soils is shown more characteristically in nitrate production than in other respects. The humus soil has a much greater nitrifying power, in respect to changing both its own organic

matter and that added to it. Probably a part of the variation is due to difference in soil flora and this in turn is due to lack of organic material in the original sandy soil. And too, if it is only in the soil films about the soil particles that organisms are active, as is probable, the greater number of particles in the humus soil would mean greater film space in which activities might occur. According to data at hand the humus soil should have a surface area of its particles just about twice that of the sandy soil, or equivalent to  $2\frac{2}{3}$  and  $1\frac{1}{3}$  acres of surface respectively. This alone is sufficient to account for the difference in activity of organisms in the two soils, though the amount of bacterial food may be an important factor as well.

The stimulation of nitrification due to the presence of lime is too conspicuous to need pointing out. There are, however, a few exceptions. Ammonium sulfate, which is physiologically acid, and casein, which is rather more acid than basic, respond quite strongly. Blood and albumin which are not predominantly acidic do not make so consistent a response to lime. And alfalfa which may be considered physiologically basic, due to the fact that upon complete oxidation a basis residue remains, responds still less. On the other hand the humus soil which is more acid by a ton and a half is much the more active soil. The indications are, therefore, that other factors may be of greater weight than is soil acidity, at least when this acidity is measured only in terms of total reactive acids present. Along with this may be taken the fact that a maximum nitrification is reached at the last sampling, which indicates that materials other than carbonates were functioning as a base, as the carbonates have been practically exhausted long before in the cases where nitrification is greatest.

Acidity is probably overemphasized sometimes, at least to the extent that lime is given too much credit from this standpoint. It is entirely probable that a neutral soil might respond to lime in a nitrification study due partly to the indirect effect of the carbonate on the soil flora or for other reasons. Aside from its neutralizing power, an important effect upon the soil flora comes indirectly through the influence of lime in improving the mechanical condition of the soil.

#### NITROGEN SUMMARY

In table 4 a summary of the nitrogen relationships is presented by taking averages and adding the ammonia and nitrate together. There is also given an average of all samplings with and without lime, showing the results after deducting the soil itself, which may be considered as a blank.

It is quite remarkable that on both soils the sum of the nitrates and ammonia is greater in most cases on the unlimed than on the limed pots where organic treatments are given, but that the reverse is true where there is no addition of organic material. These results would indicate that lime could act toward conserving the organic matter at least when fairly heavy applications were made. The ammonium sulfate-treated soils behave as the soil alone, show-

TABLE 4  
Nitrogen summary. Sum of ammonia and nitrates

	2 WEEKS			5 WEEKS			10 WEEKS			15 WEEKS			AVERAGE		
	No lime		Lime	No lime		Lime	No lime		Lime	No lime		Lime	No lime		Lime
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Humus soil															
Soil alone.....	32.9	45.2	97.9	48.7	97.9	170.9	70.3	170.9	82.2	209.7	58.5	130.9	58.5	130.9	minus soil
Albumin.....	949.9	690.8	363.9	818.3	363.9	588.1	949.4	588.1	1172.2	895.8	972.4	634.6	972.4	913.9	503.7
Casein.....	1023.7	845.6	972.3	980.9	972.3	826.6	1026.5	826.6	1114.2	1478.8	1036.3	1030.8	1036.3	977.8	899.9
Starch.....	21.7	10.5	16.8	14.0	16.8	52.0	52.0	52.5	79.4	166.0	41.8	61.4	41.8	—16.7	—69.5
Blood.....	454.8	471.9	945.2	701.1	945.2	578.6	874.3	578.6	964.3	1005.5	748.6	750.3	748.6	690.1	619.4
Dextrose.....	23.8	18.9	51.9	35.7	51.9	95.2	73.1	95.2	109.4	140.2	60.5	76.5	60.5	2.0	—54.4
Alfalfa.....	73.1	78.6	161.6	139.7	161.6	183.4	181.7	183.4	341.9	276.0	184.1	174.9	184.1	125.6	44.0
Double alfalfa.....	105.9	105.1	171.4	200.8	171.4	379.7	294.8	379.7	361.1	433.5	240.6	272.4	240.6	182.1	141.5
Ammonium sulfate.....	143.3	139.4	211.1	169.0	211.1	305.9	150.7	305.9	174.5	311.5	159.4	242.0	159.4	100.9	111.1
Sandy soil															
Soil alone.....	22.1	33.0	30.9	20.9	30.9	68.1	39.6	68.1	51.5	63.4	33.5	48.8	33.5	48.8	
Albumin.....	666.4	525.0	373.8	632.5	373.8	603.7	782.2	603.7	832.3	826.7	728.4	582.3	728.4	694.9	533.5
Casein.....	797.9	766.9	257.2	586.1	257.2	695.1	743.4	695.1	971.8	619.1	774.8	584.6	774.8	741.3	535.8
Starch.....	16.8	11.2	11.2	11.2	11.2	11.2	11.2	11.2	19.6	56.4	14.7	22.5	14.7	—18.8	—26.3
Blood.....	542.4	447.6	845.0	729.6	845.0	674.9	754.8	674.9	1033.4	745.2	777.5	678.2	777.5	744.0	629.4
Dextrose.....	14.0	11.2	11.2	11.2	11.2	14.0	19.6	14.0	58.1	57.9	25.7	23.6	25.7	—7.8	—25.2
Alfalfa.....	61.4	46.9	101.2	82.4	101.2	130.8	111.1	130.8	210.3	223.0	116.3	125.5	116.3	82.8	76.7
Ammonium sulfate.....	159.8	132.3	188.3	156.0	188.3	168.1	199.4	168.1	245.4	394.4	190.1	220.8	190.1	156.6	172.0

ing an increase of total nitrogen changed, due to the application of lime. Different results would probably be obtained under field conditions, since in practically all tests that have been reported lime has resulted in a more rapid depletion of organic matter.

The greater increase in nitrogen change on the untreated soil emphasizes the need of keeping up the organic supply of nitrogen-poor soils. The rate of change is more than doubled in many cases by the presence of the lime, which means that poor soils would be soon exhausted under such treatment. Though the absolute amount of nitrogen changed over is much greater, due to the added organic materials, the rate of change is more economical, and if conditions may be provided such that either plants or the soil organisms can take up the nitrate as produced, there is no loss. The use of cover crops would assist in securing this end.

The sandy soil seems to have converted the nitrogen of the blood somewhat more efficiently than did the humus soil. The same can be said of the ammonium sulfate on the unlimed pots. The total nitrogen changed is observed to have been greater at the first sampling than at the two succeeding ones for the albumin and casein, and then to have increased again at the last sampling. These results are probably due to the multiplication of organisms. Later the protein of their bodies was changed over again. It is, therefore, impossible to determine the proportion of total original material that may have been changed. Some doubtless passed through the cycle of changes 2 or 3 times while very resistant residues remain unattacked. As to whether the synthesized proteins of bacterial cells might be more susceptible of nitrification, there is little evidence. Claims are made both ways. Probably the correct interpretation is that some bacterial proteins are more easily changed than some original soil protein and vice versa.

Taking average figures it will be noted that about a third of the albumin nitrogen, more than half the casein nitrogen, somewhat more than a third of the blood nitrogen, less than a tenth of the alfalfa nitrogen and nearly two-thirds of the ammonium sulfate nitrogen has existed in the form of ammonia and nitrates on the humus soil in the presence of lime. Without lime more than 60 per cent of the nitrogen, less than half the nitrogen of blood, a similar proportion of the alfalfa nitrogen, and about half the ammonium sulfate nitrogen has existed in these forms. Considering maximum figures, nearly 80 per cent of the albumin, nearly 90 per cent of the casein, a little more than half the ammonium sulfate nitrogen has been changed in the humus soil. In some cases the maximum changes occur on the limed, and in others on the unlimed pots. The figures for the sandy soil would run considerably lower in most cases.

An attempt with little success was made to correlate the composition of the protein with its rate of nitrification. A difficulty lies in the fact that the composition does not tell the manner in which the different amino acids are linked together in the protein molecule. This is probably as great a factor as is the nature of the actual amino acids present.

Blood is probably over half hemoglobin, nearly a third albumin, and contains other constituents. The albumin has been studied but there is no data as to the decomposition of hemoglobin or other portions of its constituents. The gradual and regular decomposition of blood may be accounted for because of the fact that it is a mixture of proteins, some portions probably decomposing slowly and thereby tending to hold the absolute activity in check. It is known that hemoglobin resists the activity of putrifying organisms, but whether due to anti-ferments, or for other reasons is not known. The slow decomposition of the blood probably is due in part at least to the hemoglobin present.

## ACIDITY RESULTS

The acidity results are summarized in table 5. The modified Tacke Method (19) as previously described, was used. Briefly this method consists in bringing the acid soil into contact with pure calcium carbonate, using water as the contact medium. After thorough shaking and aeration for 10 hours the carbon dioxide liberated by the acid soil is titrated and the result is considered the lime requirement of the soil. The data is presented in terms of tons of calcium carbonate per acre.

TABLE 5

*Lime requirement of the variously treated soils, in tons per 2,000,000 pounds of soil*

	2 WEEKS	5 WEEKS	10 WEEKS	15 WEEKS	MORE OR LESS THAN THE SOIL ALONE			
					1	2	3	4
<b>Humus soil</b>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>
Soil alone.....	4.60	4.10	4.10	4.00				
Albumin.....	2.16	3.35	2.60	2.85	-2.44	-0.75	-1.50	-1.15
Casein.....	2.45	3.95	3.95	3.90	-2.15	-0.15	-0.15	-0.10
Starch.....	4.55	3.85	4.10	3.55	-0.05	-0.25	0.00	-0.45
Blood.....	3.85	3.90	4.00	3.70	-0.75	-0.20	-0.10	-0.30
Dextrose.....	3.85	3.95	4.45	3.95	-0.75	-0.15	+0.35	-0.50
Alfalfa.....	4.10	3.95	4.30	4.05	-0.50	-0.15	+0.20	+0.05
Double alfalfa.....	4.10	4.00	4.15	4.50	-0.50	-0.10	+0.05	+0.50
Ammonium sulfate.....	4.80	4.75	5.10	5.45	+0.20	+0.65	+1.00	+1.45
<b>Sandy soil</b>								
Soil alone.....	3.05	2.50	2.60	2.65				
Albumin.....	1.30	1.25	1.35	2.40	-1.75	-1.25	-1.25	-0.25
Casein.....	1.50	2.40	1.65	2.15	-1.55	-0.10	-0.95	-0.50
Starch.....	2.60	2.50	2.15	2.55	-0.45	0.00	-0.45	-0.10
Blood.....	1.95	2.85	2.30	2.85	-1.10	+0.35	-0.30	+0.20
Dextrose.....	2.85	2.55	2.25	2.95	-0.20	+0.05	-0.35	+0.30
Alfalfa.....	2.80	2.70	2.25	2.40	-0.25	+0.20	-0.35	-0.25
Ammonium sulfate.....	2.95	3.15	3.25	3.25	-0.10	+0.65	+0.65	+0.60

The albumin and the casein have acted very much as an application of lime at first. This could be explained as due partly to the rapid production of

ammonia without accompanying nitrification to increase acidity. The albumin fluctuates (partly perhaps due to errors of sampling) more than does the casein, but causes greater reduction in lime requirement at the first sampling, and much greater reduction in succeeding samplings. This is not in accordance with the predominantly acidic nature of casein, and indicates decarboxylation or oxidation of acidity. Starch and dextrose have likewise, though to a smaller extent, reduced the acidity, except at the third sampling on the humus soil. On the sandy soil both carbohydrates have had a very fluctuating effect, and could scarcely be given a preponderance of credit either way. It is probable that organic acids may have oxidized faster in the presence of the dextrose and starch, and there is also the fact that nitrification does not occur to increase acidity. Blood, like the other protein materials, reduces the acidity considerably at first, but after that only to a slight degree on the humus soil, and on the sandy soil its conduct is not consistent either way. Alfalfa has first reduced the acidity and then increased it on both soils, but only to such an extent as to be insignificant. Its very complete nitrification would have the effect of increasing acidity, while the basic residue of minerals would have the opposite effect. The ammonium sulfate has caused a gradual increase in acidity on both soils, though to a less extent on the sandy soil. The data shows it to be the only material which could be definitely stated to have increased the acid reaction of the soil, excluding nitric acid itself, which is rather strong evidence that mineral acids are much more likely to act deleteriously. Several of the other compounds produce much more nitric acid, and any of them are potentially capable of producing organic acids, but since no other treatment has an appreciable sulfuric acid residue, the major effect must be attributed to this.

#### ACIDITY RELATIONSHIPS

The relation between soil acidity and the activity of soil flora is a somewhat complicated problem. As the soil reaction changes, group relations of soil organisms must shift. This is especially true since a high concentration of hydrogen-ion which is the true acidity, is deleterious to many bacteria, such as the nitrate producers, and azofiers but is beneficial to certain fungi, more especially the molds.

There is also undoubtedly a marked adaptation of the flora to soil reaction. Acidity must naturally result through a cumulative process, and as the intensity increases a more and more acid resistant strain of organisms of any species would seem natural. Such an adaptation would be comparable to that purposely brought about in the preparation of vaccines by the producing of strains of pathogenic organisms of weak virulence. Not only are the physiological, but likewise the morphological characters, very much subject to modification. Though there has never been a demonstration to the effect that a more efficient strain of organisms, such as the nitrifiers, may be pro-



duced, the cause may be attributed largely to the lack of methods. In fact, the organisms may be less efficient, but more resistant to acidity. Accurate methods, unless it be the hydrogen electrode method little used in soil studies, are not available for determining the active acidity of the soil. The ideal plan for such a study would be to start with a neutral soil, and by the use of artificial acids to prepare soils of definite hydrogen-ion concentration increasing by succeeding degrees to a high intensity. Then a study, where both pure and mixed strains of organisms were used should detect any adaptation, which might occur through perhaps several months time.

From its effect upon the activity of organisms, the question may be asked, how does acidity occur in the soil? It is seldom, at least, that a titrable acidity can be extracted from soils, yet the same soils turn litmus paper, and give other indications of a functioning acidity. Acids are not active until ionized and not ionized until dissolved. Therefore, there must be a soluble acidity, even though it cannot be extracted. Buffering would, of course, explain the fact that a soil extract is neutral to indicators. In fact, by mixing mono- and di-sodium phosphates a neutral solution may be prepared, which would require the addition of 0.5 equivalent of HCl or NaOH before the reaction would be acid to congo red, or alkaline to phenolphthalein. This buffer action may be brought about by various acid salts, amphoteric substances, salts of weak acids and bases, etc., and undoubtedly some soils possess a large reserve acidity or alkalinity. But it seems also that it is not possible to extract a buffered solution from the soil (a number of tests were made on these treated soils) as indicated by incapacity of the extract to decompose calcium carbonate. Yet both artificially prepared buffered solutions and acid soils decompose carbonate easily. It would be of interest to know whether an oil pressure extract from acid soils might not possess a buffer action.

That many soil acids, including those of both organic and mineral nature, are quite insoluble and inactive for that reason is likewise quite without question. Taking all of these facts together it is easy to see that the absolute acidity of a soil may not have a very definite significance, since it is measured by the total amount of base taken up, and gives no indication as to what portion of the measured value may be due to reserve acidity and relatively insoluble acids. The reserve acidity is probably of little or no direct injury to either plants or organisms, though it is likely to use up the carbonates applied to the soil.

The evidence taken together is strong, therefore, for a soluble acidity, but just how and where it exists is not so easy to say. From the different reactions given the conclusion that such soluble or active acids as are present exist only in the soil film, would not be irrational. It is probable that only the film water functions in the nutrition of plants or is active in promoting the activity of soil organism. It is consistent with the nature of protein substances and organic matter in the humified state that they should form a colloidal film about the mineral particles of the soil, binding the water

as a colloidal hydrate. The force which holds this film is difficult to measure, but it has been estimated at from 6,000 to 25,000 atmospheres. If organisms are active, principally in this film, and plant roots feed from the same film water, then there is an intimate relationship, on the one hand, between the reaction of the soil, which may perhaps not extend its sphere beyond the film thickness, and bacterial activity, and on the other hand between soil reaction and crop production. Such intimate relationships make it possible for the growing crop to exert considerable influence upon the changes brought about by the organisms and vice versa. Root hairs cling to soil particles, and organisms likewise are separated from the particles only with considerable effort, which is more evidence of a closely existing relationship such as has been described. These are not essentially new ideas but are a somewhat modified interpretation. Soil acidity, though without adequate reason perhaps, has been attributed to absorption forces, be they chemical or physical, which are probably somewhat similar to the force which binds water in colloids and which holds the films about particles. Nevertheless acidity to the degree that it is soluble may exist in the soil film and yet not be an adsorption phenomenon as popularly interpreted. It would not be presumed that all acidity or all colloidal matter would exist in films, though the active portion might be limited to such a sphere of functioning. Any which long existed in the free state would naturally be washed from the soil in the drainage.

Plummer (15), by the use of the hydrogen electrode, decided that soils vary appreciably in the hydrogen-ion concentration of the soil suspension. Similar results were obtained by Sharp and Hoagland (16). Plummer (15) worked also on the soil film water, which was obtained by Morgan's oil pressure method. It is significant that he found the reaction of this film water to be qualitatively the same as that of a soil suspension. The concentration of acidity or alkalinity was much greater, however, in the film water. The general conclusion of these workers is that an acid soil must always present an acid solution to its plants.

Another point of interest in the interpretation of these studies is the effect of the nitrification process upon the soil reaction. The nitric acid produced makes heavy drains upon carbonates, as the data shows, the amount of base used being somewhat, if not entirely, proportional to the nitrate. At the same time not only the ammonia but other nitrogen bases,<sup>4</sup> including the various amines, choline, guanidine, purine, and pyrimidine bases, etc., serve in the same way. Some nitrogen bases are stronger even than sodium hydroxide, and though no definite measures can be made, the organic acids produced, may not be any more significant than are bases liberated. The

<sup>4</sup> A nitrogen base results whenever a compound possesses nitrogen which may change in valence from 3 to 5. In water solution this change may result in the formation of an hydroxide as in the case of ammonia. In other cases the nitrogenous compound may be presumed to combine directly with the acid. If it were hydrochloric acid, an hydrochloride would be formed.

data in a general way confirms this idea, though a basic material would probably be nitrified under conditions where one predominantly acid would not.

In fact Funchess (7) of Alabama found that certain nitrogen bases, such as quinoline and guanidine carbonate, were nitrified best in acid soils. The effect was marked to the degree that lime prevented the decomposition of such materials. Other nitrogen bases, however, nitrified in the presence of lime, some more rapidly than in acid soil.

Another point related to the interpretation of the data is the effect of the original materials on the soil reaction. Since proteins have a reserve acidity, they might be expected to decompose calcium carbonate. But the addition of the same amount of proteins as was added to the soil in the studies produced no measurable effect upon the lime requirement. This may be explained if in the original protein material there is an internal salt formation, due to the free carboxyls neutralizing the free amino groups. It is thought that such a ring formation does exist. It would seem, therefore, that some chemical or enzymatic action were necessary to break the ring formation and start decomposition before a measurable effect is produced on the soil reaction. The data indicates that such changes occur rather rapidly, however, as maximum effects are produced at the first sampling.

#### RESIDUAL CARBONATES

The residual carbonates were determined with the same apparatus used to determine the acidity, using phosphoric acid (strength 1 to 15) to decompose the carbonate. Blanks were always run on the untreated soil and subtracted from the results. The blank is nearly always low, about 1 cc. and did not differ greatly on the variously treated pots. The results are shown in table 6 expressed in tons per acre.

The data show again that albumin, casein, and likewise to a lesser degree, blood show a protective action for the lime at the first sampling. Since these materials act as a base in both acidity and carbonate determinations, as is evident, it would seem that decarboxylation must have occurred rather rapidly. This would of course always leave a basic residue. Doubtless too, the ammonia again would tend to preserve mineral bases of the soils. These conclusions are further justified from the fact that though casein is naturally predominantly acidic, it renders greater protective action than any of the other treatments. This again would indicate that much organic acidity might be rather ephemeral than significant. After the first sampling the drain upon the carbonates with these same treatments becomes marked. This is apparently due to the fact that nitrification has begun, thereby not only removing the basic action of the ammonia, but increasing the acidity by producing nitric acid. The changing of 28 pounds of ammonia nitrogen to the nitric form may theoretically increase the lime requirement by 200 pounds, due to the double action of removing base and producing acidity. This alone might

account for a considerable change of reaction. Blood and casein produce somewhat more violent effects in using up carbonate than does the albumin. On the sandy soil the blood produces very strong effects. Starch and dextrose have not caused marked effects, but have had a tendency to use up carbonates. It will be remembered that they had a tendency also to decrease acidity, so that taken together, their application is without significance in effect upon soil reaction. At the third sampling dextrose on the sandy soil has caused a marked drain upon carbonates but the high figures are probably due to error. The alfalfa treatment, likewise, has had no consistent effect upon the supply of carbonates, which might be predicted from its nature.

TABLE 6  
*Residual carbonates on treated soils at the several samplings*  
Expressed in tons per acre

	1	2	3	4	MORE OR LESS THAN SOIL UNTREATED			
					1	2	3	4
<b>Humus soil</b>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>	<i>tons</i>
Soil alone.....	2.75	2.50	1.65	1.90				
Albumin.....	4.65	1.00	0.90	0.00	+1.90	-1.50	-0.75	-1.90
Casein.....	6.10	0.50	0.65	0.05	+3.35	-2.00	-1.00	-1.85
Starch.....	2.65	2.00	2.45	1.80	-0.10	-0.50	+0.80	-0.10
Blood.....	3.45	0.75	0.55	0.15	+0.70	-1.75	-1.10	-1.75
Dextrose.....	3.15	2.15	1.60	1.55	+0.40	-0.35	-0.05	-0.35
Alfalfa.....	3.35	2.60	2.70	1.75	+0.60	+0.10	+1.05	-0.15
Double alfalfa.....	2.60	1.85	1.90	1.60	-0.15	-0.65	+0.25	-0.30
Ammonium sulfate.....	1.65	1.05	0.75	0.80	-1.10	-1.45	-0.90	-1.10
<b>Sandy soil</b>								
Soil alone.....	3.45	2.70	3.05	2.75				
Albumin.....	5.20	2.75	2.65	1.35	+1.75	+0.05	-0.40	-1.40
Casein.....	5.75	1.80	0.50	1.20	+2.30	-0.90	-2.55	-1.55
Starch.....	3.40	2.65	2.95	3.30	-0.05	-0.05	-0.10	+0.55
Blood.....	3.95	0.75	0.40	0.35	+0.50	-1.95	-2.65	-2.40
Dextrose.....	3.05	2.15	2.00	2.40	-0.40	-0.55	-1.05	-0.35
Alfalfa.....	3.35	2.50	2.05	2.75	-0.10	-0.20	-1.00	0.00
Ammonium sulfate.....	2.65	1.60	0.95	1.40	-0.80	-1.10	-2.10	-1.35

Ammonium sulfate, true again to its physiologically acid nature, has been just as consistent in making a drain upon the carbonates as in increasing the soil acidity, which is more evidence of the significance of mineral acids. The two soils behave in a similar manner qualitatively, but there are usually more marked effects produced upon the humus soil.

#### GENERAL DISCUSSION

The acidities and carbonates have been run in duplicate on duplicate pots and as a rule checks were good. Relative results are shown in each table in

columns to the right marked with a plus or minus sign, according to the effect produced as compared with the soil alone. The soil itself in each case has apparently become less acid after the first sampling, which might signify oxidation of organic acids present in the original soil. It is true, however, that many of the minor variations are considered accidental rather than fundamental. There are errors in mixing the original soil, and again in adding the treatments, and yet again in taking a comparatively small sample from so great a bulk of soil. Results are, therefore, interpreted with these limitations in mind.

Rather than have conclusions drawn from so brief and limited a study some general indications may be pointed out. In the first place the more acid soil has been more active in every respect, indicating that measurable acidity is not a correct index to toxicity. And again no effect which may be attributed to acids has been permanent and consistent except where a mineral acid could be credited for the cause. These permanent effects have resulted from the sulfuric acid liberated from the ammonium sulfate and from the nitric acid accruing from nitrification. Where only organic acids may be presumed to have appeared the effect has been slight or, if temporarily quite appreciable, has soon disappeared. Another effect may be noted in that the basicity resulting from the breaking down of protein is more permanent than any acidity which may result. This is due to the fact that acids are either volatile or are oxidized to carbon dioxide which is volatile. Nitrogenous bases, on the other hand, are continuously active being absorbed and held by the soil until nitrified. In general, then, organic matter seems to act the part of a base rather than of acid, at least to the extent that a given acidity is less detrimental in the presence of an adequate supply of organic materials. Even though organic acids may temporarily take up bases, they are freed again by the complete oxidation of the organic radical.

Theoretically, hydrolysis of 10 tons glucose or starch may produce an acidity equivalent to 5.6 tons of calcium carbonate, if only acetic acid were produced and the charge were complete. It is perfectly evident that nothing like this effect ever occurs. In the same way the change of the one ton of ammonium sulfate, if it were complete would cause an acidity of 1.5 tons, which is only a little more than is actually found in some cases. Likewise the nitrogen of the nitrogenous materials should produce in round numbers about 5.3 tons acidity if the change were complete. But since some ammonia is always present which has neutralizing power and the nitrogen change is never complete, a much smaller effect in increased acidity is produced. The nitrogen of the alfalfa if all changed to nitric acid would produce only about a ton of acidity, while the lime from the same source would be equivalent to nearly half a ton, so that no marked effects would be expected from alfalfa as the data shows. Therefore, from combined data both experimental and theoretical, we may conclude that the addition of organic materials to soils is not likely to have a very decided effect in increasing acidity. In fact, the humified

organic matter may act as a protector of base, by combining with it to form insoluble and non-leachable compounds. Then as oxidation proceeds, the mineral bases thus preserved assist in neutralizing any harmful acidity that may occur. Additional base must be supplied, however, to satisfy the demands of the nitric, sulfuric, or other mineral acids, which may at times tend to accumulate, partly due to the use of physiologically acid fertilizers.

#### PHYSICAL EFFECTS OF TREATMENTS

There is a physical effect of these different treatments upon the soils which is worthy of note. The albumin and casein pots required less water than the others, due to the failure to evaporate moisture as rapidly. This is in accordance with the nature of colloidal material, such as the various proteins, to bind or hold water. Casein, especially in the presence of alkali, has been found to show marked hydrophylic properties. There is also a tendency for the formation of a grayish incrustation on the pots, and the soil handles poorly, is hard and lumpy, even in the presence of lime. These materials, and also blood-treated soils, filter poorly in the early part of the experiment. Colloidal material passed 2 thicknesses of filter paper. Later blood recovered from this characteristic and gave a clear filtrate. These same materials, especially in the early work, gave a filtrate of a somewhat greenish-yellow color, due doubtless to some organic decomposition product.

Of the two, the humus soil worked rather better, giving a clearer filtrate. The carbohydrate materials on both soils caused marked physical effects, though producing a different condition from that of the proteins. The starch, in particular, caused the soil to remain wet and sticky, of a consistency similar to putty. Starch pots too, although apparently of the same water content, always showed a higher per cent of moisture. The alfalfa-treated pots were in the best physical condition, remaining granular, and in good tilth. The ammonium sulfate had little effect. This factor may be worthy of consideration, therefore, in that the physical properties of a soil depend very much upon the nature of organic materials and fertilizers added, and that such factors in turn greatly influence bacterial activity and the producing power of a soil. The evil effects of some treatments are not overcome even by lime, when added in amounts compatible with practicability.

#### SUMMARY

1. None of the organic treatments has increased the lime requirement of the soils. The highly nitrogenous materials have rather had the effect of decreasing the acidity. This effect is very marked at the first sampling.
2. Ammonium sulfate, on the other hand, has consistently caused a marked increase in the lime requirement of both soils.
3. The carbohydrate materials have had a small and inconsistent effect upon the soil reaction.

4. The highly nitrogenous organic materials which diminished the acidity tended to protect the carbonates of the soil at the first sampling. Later the same materials used up the limestone quite completely in some cases and to a much greater extent in all cases than did the soil alone.

5. Ammonium sulfate, likewise, very consistently exhausted the lime of the treated soils.

6. The carbohydrates, as is true of the alfalfa also, had no more marked effects in exhausting the carbonates than in increasing the acidity.

7. Ammonification is greater in the absence of lime on both acid soils. A difference in the soil flora is a possible explanation. The ammonification is probably due in part to the activity of molds.

8. Both casein and albumin ammonify more rapidly than does blood which is of higher nitrogen content.

9. Ammonia does not accumulate in the presence of either carbohydrates or alfalfa.

10. Nitrification occurs most rapidly in the presence of lime.

11. Nitrification is slow in starting in the presence of the nitrogenous materials, except blood, probably due to a slight toxicity of accumulated ammonia and exhaustion of nitrates caused by the multiplied flora.

12. No nitrates were found in the presence of the carbohydrates until the end, probably because they were consumed by the organisms of the soil.

13. Taking the sum of nitrates and ammonia there is the greatest action in the presence of lime on the untreated soil, but the reverse is true in most cases for the treated pots.

14. The two soils show as marked differences in their behavior as do the different treatments. The more acid soil is much more active, probably because of greater content of organic matter and a more abundant flora.

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## SOLUBLE NON-PROTEIN NITROGEN OF SOIL

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Sometime ago there was published from this laboratory a description of a method (3) for the determination of the soluble non-protein nitrogen of the soil. Since that time the method has been considerably improved and many determinations have been made with the refined method. It is the object of this paper to report this work. An experiment has been started whose direction the senior author of this paper must discontinue, and, while yet incomplete many results have been obtained which are interesting and valuable and should go on record.

### DEVELOPMENT OF THE METHOD

Early in the work it was found that from 96 to 99 per cent of the nitrogen extracted from normal soil by 1 per cent hydrochloric acid, belonged to the class of soluble non-protein nitrogen compounds. Since the small amount of protein nitrogen contained in the acid extract was insignificant when compared to the total soluble non-protein nitrogen, no appreciable error was introduced by its complete inclusion in this group of compounds.

The usual procedure in the old method for the determination of humus was to extract the soil with 1 per cent hydrochloric acid until the wash water from the soil contained no calcium. With that in mind, experiments were planned to determine the proper number of extractions in order to obtain a maximum amount of soluble non-protein nitrogen. Accordingly, two acid and two basic soils were extracted various numbers of times with acid, and total soluble non-protein nitrogen was determined. For the details of this the preceding paper on this subject is referred to. Briefly it consists of extracting the acid-extracted soil with 1.5 to 1.75 per cent sodium hydroxide and the clarification of this extract in a centrifuge running at a speed of 30,000 revolutions per minute. The clear extract is neutralized with sulfuric acid, and then acidified with sufficient tri-chloroacetic acid to give finally 2.5 per cent of the latter acid. After filtration total nitrogen is determined in an aliquot of the filtrate, by the micro method (2). The nitrogen, so found, is added to that found in the acid extract.

The results and further remarks will be found in table 1.

Duplicate determinations were made on all the soils but are not included in the table. The duplicates agreed very well, the average derivation from

the mean in all the above experiments being less than 0.0004 gm. nitrogen per 100 gm. soil.

Considering the two acid soils it is observed that acid extraction did not increase the amount of soluble non-protein nitrogen to an appreciable extent. The results for the Carrington silt loam are rather inconsistent. This was

TABLE 1

*Soluble non-protein nitrogen from soils extracted various numbers of times with 1 per cent hydrochloric acid*

Results expressed as gm. nitrogen per 100 gm. air dry soil

SOIL	NO. OF ACID EXTRACT	TEST FOR Ca	SOLUBLE NON-PROTEIN NITROGEN		
			In acid extract	In alkali extract	Total
			gm.	gm.	gm.
Calhoun silt loam acid soil with lime requirement 4.5 tons $\text{CaCO}_3$	None			0.0333	0.0333
	2	Trace?	0.0045	0.0282	0.0327
	4	Absent	0.0045	0.0280	0.0325
	6	Absent	0.0048	0.0280	0.0328
	8	Absent	0.0048	0.0282	0.0330
Carrington silt loam acid soil with lime requirement 5 tons $\text{CaCO}_3$	None			0.0371	0.0371
	2	Trace	0.0034	0.0394	0.0428
	3	Trace	0.0038	0.0375	0.0413
	4	Absent	0.0039	0.0371	0.0410
	5	Absent	0.0040	0.0351	0.0391
	6	Absent	0.0041	0.0357	0.0398
	7	Absent	0.0041	0.0352	0.0393
	8	Absent	0.0041	0.0340	0.0381
Fargo loam basic soil 26.3 tons $\text{CaCO}_3$	None			0.0364	0.0364
	2	Very heavy	0.0045	0.0302	0.0347
	4	Heavy	0.0049	0.0347	0.0396
	6	Light	0.0056	0.0404	0.0460
	8	Trace	0.0056	0.0407	0.0463
Marshall silt loam basic soil 4.8 tons $\text{CaCO}_3$	None			0.0216	0.0216
	2	Light	0.0025	0.0262	0.0287
	4	Trace	0.0028	0.0278	0.0306
	6	Absent	0.0028	0.0280	0.0308
	8	Absent	0.0028	0.0277	0.0305

the first soil experimented with and the duplicates did not agree as closely as in the remainder of the work.

The results for the soils containing lime show conclusively that in order to obtain all of this class of compounds, acid extraction must be carried to the point where but little, if any calcium can be found in the wash water.

The next point taken up in the refinement of the method was to find out whether nitrates would be determined by this method or whether they would

be entirely eliminated. In a paper published by the senior author about simultaneously with this report it was shown that the ordinary methods for total nitrogen in soils, when used on soil containing 0.6 per cent or more of organic carbon, included the nitrogen of nitrates, while with soils containing less organic matter the nitrates were only partially included. It was suspected, therefore, that the nitrate nitrogen in the acid extract might be only partially included in the total nitrogen determination. An experiment was carried out to test this point. It consisted simply of adding a known amount of nitrate solution to an acid extract of a soil and then digesting it as for total nitrogen. A slight increase in total nitrogen was obtained but not nearly sufficient to account for all the nitrogen added. An effort was then made to reduce all the nitrate nitrogen to ammonia by the addition of iron to the acid solution. In this, success was only attained by the use of excessive amounts of iron, 6 gm. of iron reduced 6 mgm. of nitrate nitrogen completely to ammonia, but 7 gm. failed to reduce 12 mgm. of nitrate nitrogen. Since such large amounts of iron would seriously interfere with the subsequent digestion for total nitrogen, the use of iron was abandoned.

Because of the success Allen (1) has had in the determination of nitrates by the use of Devarda alloy in alkaline solution it was decided to attempt to modify that method for the purpose of this work. After considerable preliminary work the following procedure, which was found to include all nitrate nitrogen, was adopted. The acid extract, with the wash water, which altogether usually occupies a volume of about 600 cc. is made slightly alkaline, to the extent of 2 cc. of saturated sodium hydroxide. Then 2 gm. of devarda alloy is added, the mixture distilled through block tin condensers into standard acid until the residue has a volume of 150 cc. This is transferred to 500 cc. Kjeldahl flasks and then digested in the usual manner for total nitrogen. The nitrogen so obtained plus that from the first distillation gives, of course, the total nitrogen in the acid extract. This method while rather cumbersome has been found the only reliable method for the total nitrogen of acid extracts of soil.

After the method had been perfected as detailed above, it was decided to investigate the non-protein nitrogen on some of the samplings of the soils from the experiment described by Stephenson in the preceding paper. The original plan was to determine this group of compounds in the second and last sampling but it was necessary to discontinue the work after the completion of the analysis of the soils from the second sampling. In the first paper on this method it was shown that carefully purified protein, when added to soil, did not increase the soluble non-protein nitrogen if the soil was immediately subjected to the analysis. Since the commercial preparation of the proteins was used in this pot experiment it was thought best to determine whether the soluble non-protein fraction was increased by these commercial preparations. Also the amount of soluble non-protein nitrogen in the original soil mixtures of all the other materials containing nitrogen, before any decomposition had taken place, was determined.

TABLE 2  
*Carrington silt loam*  
 All results are expressed in grams per 100 gm. air-dry soil

SOIL NUMBER	TREATMENT	NON-PROTEIN NITROGEN										NH <sub>4</sub> NITROGEN + NO <sub>3</sub> NITROGEN		UNKNOWN NON-PROTEIN NITROGEN	
		In HCl extract		Gm. in alkali extract		Total non-protein nitrogen		Original	After 5 weeks	gm.	gm.	Original	After 5 weeks	gm.	gm.
		Original	5 weeks inclusive	Original	5 weeks inclusive	Original	gm.								
1 and 2	nothing.....	0.0037	0.0063	0.0395	0.0349	0.0432	0.0412	0.0027	0.0049	0.0405	0.0363	0.0027	0.0049	0.0405	0.0363
3 and 4	7 tons lime.....	0.0037	0.0101	0.0395	0.0300	0.0432	0.0401	0.0027	0.0103	0.0405	0.0298	0.0027	0.0103	0.0405	0.0298
5 and 6	10 tons egg albumen.....	0.0087	0.0835	0.0399	0.0454	0.0486	0.1289	0.0027	0.0818	0.0459	0.0471	0.0027	0.0818	0.0459	0.0471
7 and 8	10 tons egg albumen and 7 tons lime.....	0.0087	0.0330	0.0399	0.0411	0.0486	0.0741	0.0027	0.0364	0.0459	0.0377	0.0027	0.0364	0.0459	0.0377
9 and 10	10 tons casein.....	0.0053	0.1066	0.0413	0.0417	0.0466	0.1483	0.0027	0.0981	0.0439	0.0502	0.0027	0.0981	0.0439	0.0502
11 and 12	10 tons casein and 7 tons lime.....	0.0053	0.0527	0.0413	0.0349	0.0466	0.0876	0.0027	0.0569	0.0439	0.0307	0.0027	0.0569	0.0439	0.0307
13 and 14	10 tons starch.....	0.0037	0.0026	0.0395	0.0321	0.0432	0.0347	0.0037	0.0014	0.0405	0.0333	0.0037	0.0014	0.0405	0.0333
15 and 16	10 tons starch and 7 tons lime.....	0.0037	0.0021	0.0395	0.0306	0.0432	0.0327	0.0027	0.0017	0.0405	0.0310	0.0027	0.0017	0.0405	0.0310
17 and 18	10 tons dried blood.....	0.0042	0.0741	0.0389	0.0423	0.0431	0.1164	0.0027	0.0702	0.0404	0.0462	0.0027	0.0702	0.0404	0.0462
19 and 20	10 tons dried blood and 7 tons lime.....	0.0042	0.0603	0.0389	0.0376	0.0431	0.0979	0.0027	0.0567	0.0404	0.0412	0.0027	0.0567	0.0404	0.0412
21 and 22	10 tons dextrose.....	0.0037	0.0049	0.0395	0.0312	0.0432	0.0361	0.0027	0.0036	0.0405	0.0325	0.0027	0.0036	0.0405	0.0325
23 and 24	10 tons dextrose and 7 tons lime.....	0.0037	0.0061	0.0395	0.0285	0.0452	0.0346	0.0027	0.0052	0.0405	0.0294	0.0027	0.0052	0.0405	0.0294
25 and 26	10 tons alfalfa.....	0.0073	0.0165	0.0392	0.0331	0.0465	0.0496	0.0027	0.0140	0.0438	0.0356	0.0027	0.0140	0.0438	0.0356
27 and 28	10 tons alfalfa and 7 tons lime.....	0.0073	0.0163	0.0392	0.0297	0.0465	0.0460	0.0027	0.0162	0.0438	0.0289	0.0027	0.0162	0.0438	0.0289
29 and 30	20 tons alfalfa.....	0.0109	0.0253	0.0395	0.0323	0.0504	0.0576	0.0027	0.0209	0.0477	0.0367	0.0027	0.0209	0.0477	0.0367
31 and 32	20 tons alfalfa and 7 tons lime.....	0.0109	0.0215	0.0395	0.0312	0.0504	0.0527	0.0027	0.0172	0.0477	0.0355	0.0027	0.0172	0.0477	0.0355
33 and 34	1 ton (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.0240	0.0185	0.0389	0.0303	0.0629	0.0488	0.0222	0.0169	0.0397	0.0319	0.0222	0.0169	0.0397	0.0319
35 and 36	1 ton (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and 7 tons lime.....	0.0240	0.0104	0.0389	0.0276	0.0629	0.0380	0.0222	0.0211	0.0397	0.0169	0.0222	0.0211	0.0397	0.0169

The results first obtained by the analyses of some of these original mixtures were so much higher than the results obtained from the soils alone that it was immediately suspected that the hydrochloric acid had extracted some protein material which, of course, would be included in the soluble non-protein nitrogen by the regular procedure. The work was, therefore, repeated, and the hydrochloric acid extract as soon as obtained was nearly neutralized with sodium hydroxide and then evaporated down to about 100 cc. under diminished pressure. It was then precipitated by the addition of sufficient trichloroacetic acid to give a final strength of 2.5 per cent. The precipitate was filtered and washed with 2.5 per cent trichloroacetic acid and the nitrogen determined in the filtrate. This, then, taken as the soluble non-protein nitrogen in the original mixtures, together with that after the 5 weeks incubation in the greenhouse, is given in table 2. Also in this table the *sum* of the nitrate and ammonia nitrogen is given. These results are taken from Stephenson's paper. Since all of the ammonia and nitrate nitrogen is found in the soluble non-protein fraction, the "unknown soluble non-protein" nitrogen has been computed and appears in the last two columns of the table.

In a consideration of the results appearing in table 2 perhaps the most important sets of figures to consider are those in the last two columns, namely what has been called the "unknown soluble non-protein nitrogen." All that we know definitely of this group of compounds is that it is not ammonia or nitrates nor is it protein material. No doubt, part of it belongs to the group of amino acids and also some to the polypeptides. But little is known as to where in the di-, tri-, tetra-peptide, etc. this method draws a line. As a matter of fact there is probably no definite place. The point where precipitation takes place depends no doubt upon the amino acids in the polypeptide, as well as upon the number of amino acids.

In a consideration of the unknown soluble non-protein nitrogen two things are of interest; first, the variation of the different treatments due to the incubation, and second, variation of the results with the different treatments. Perhaps the most noteworthy thing to be seen is that the unknown soluble non-protein nitrogen has decreased during the incubation in every case except in those soils treated with the highly nitrogenous material, albumin, casein and dried blood. Even in the limed soils treated with albumin and casein there is a decrease. In the exceptions noted there is a sufficiently large increase to show there has been a quite different action in these soils. From Stephenson's results in the preceding paper it is seen that in soils 5 and 6 and 9 and 10 the amounts of ammonia are very high while nitrification has not yet started very vigorously. In the corresponding limed pots of the albumin- and casein-treated soils nitrification has proceeded much further and the fact that the soluble non-protein nitrogen in these limed soils is so low means, not that there has been less action, but that there has taken place to a greater extent the assimilation of nitrates and other simple compounds, indicating perhaps that action has been considerably more vigorous here. The

TABLE 3

*Carrington sandy loam*

All results are expressed in grams per 100 gm. of air-dry soil

SOIL NUMBER	TREATMENT	NON-PROTEIN NITROGEN				TOTAL NON-PROTEIN NITROGEN				NH <sub>4</sub> NITROGEN + NO <sub>3</sub> NITROGEN				NON-PROTEIN NITROGEN NOT KNOWN			
		In HCl extract		In alkali extract		Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks	Original	After 5 weeks
		gm.	gm.	gm.	gm.												
1 and 2	Nothing.....	0.0028	0.0042	0.0315	0.0316	0.0343	0.0358	0.0011	0.0022	0.0332	0.0336	0.0332	0.0336	0.0332	0.0336	0.0332	0.0336
3 and 4	6 tons lime.....	0.0028	0.0056	0.0315	0.0272	0.0343	0.0328	0.0011	0.0031	0.0332	0.0297	0.0332	0.0297	0.0332	0.0297	0.0332	0.0297
5 and 6	10 tons egg albumen.....	0.0042	0.0552	0.0350	0.0350	0.0392	0.0902	0.0011	0.0621	0.0381	0.0281	0.0381	0.0281	0.0381	0.0281	0.0381	0.0281
7 and 8	10 tons egg albumen and 6 tons lime.....	0.0042	0.0476	0.0350	0.0300	0.0392	0.0776	0.0011	0.0382	0.0381	0.0394	0.0381	0.0394	0.0381	0.0394	0.0381	0.0394
9 and 10	10 tons casein.....	0.0031	0.0511	0.0344	0.0345	0.0375	0.0856	0.0011	0.0579	0.0364	0.0277	0.0364	0.0277	0.0364	0.0277	0.0364	0.0277
11 and 12	10 tons casein and 6 tons lime.....	0.0031	0.0418	0.0344	0.0300	0.0375	0.0718	0.0011	0.0254	0.0364	0.0464	0.0364	0.0464	0.0364	0.0464	0.0364	0.0464
13 and 14	10 tons starch.....	0.0028	0.0038	0.0315	0.0289	0.0343	0.0327	0.0011	0.0011	0.0332	0.0316	0.0332	0.0316	0.0332	0.0316	0.0332	0.0316
15 and 16	10 tons starch and 6 tons lime.....	0.0028	0.0028	0.0315	0.0270	0.0343	0.0298	0.0011	0.0011	0.0332	0.0287	0.0332	0.0287	0.0332	0.0287	0.0332	0.0287
17 and 18	10 tons dried blood.....	0.0033	0.0652	0.0323	0.0365	0.0356	0.1017	0.0011	0.0736	0.0345	0.0281	0.0345	0.0281	0.0345	0.0281	0.0345	0.0281
19 and 20	10 tons dried blood and 6 tons lime.....	0.0033	0.0637	0.0323	0.0317	0.0356	0.0954	0.0011	0.0498	0.0345	0.0456	0.0345	0.0456	0.0345	0.0456	0.0345	0.0456
21 and 22	10 tons dextrose.....	0.0028	0.0032	0.0315	0.0269	0.0343	0.0301	0.0011	0.0011	0.0332	0.0290	0.0332	0.0290	0.0332	0.0290	0.0332	0.0290
23 and 24	10 tons dextrose and 6 tons lime.....	0.0028	0.0037	0.0315	0.0266	0.0343	0.0303	0.0011	0.0011	0.0332	0.0292	0.0332	0.0292	0.0332	0.0292	0.0332	0.0292
25 and 26	10 tons alfalfa.....	0.0056	0.0094	0.0312	0.0274	0.0368	0.0368	0.0011	0.0083	0.0357	0.0287	0.0357	0.0287	0.0357	0.0287	0.0357	0.0287
27 and 28	10 tons alfalfa and 6 tons lime.....	0.0056	0.0117	0.0312	0.0240	0.0368	0.0357	0.0011	0.0101	0.0357	0.0256	0.0357	0.0256	0.0357	0.0256	0.0357	0.0256
29 and 30	1 ton (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.0235	0.0175	0.0312	0.0257	0.0547	0.0432	0.0217	0.0156	0.0330	0.0276	0.0330	0.0276	0.0330	0.0276	0.0330	0.0276
31 and 32	1 ton (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and 6 tons lime.....	0.0235	0.0164	0.0312	0.0235	0.0547	0.0399	0.0217	0.0165	0.0330	0.0234	0.0330	0.0234	0.0330	0.0234	0.0330	0.0234

very decided decrease in unknown soluble non-protein in the soils treated with dextrose and starch means the same thing, namely, that the bacteria have used up the simpler nitrogen compounds because of the presence of such large amounts of available energy-producing material.

The results for the more sandy soil appear in table 3.

Upon an inspection of the results in table 3 it is observed that, in general, they are similar to those of the soil higher in organic matter except in an important and striking instance. In the soils treated with the highly nitrogenous materials in the case of the sandy soil, the unlimed soils show a decrease in unknown soluble non-protein nitrogen while the limed show an increase. The opposite was true for the humus soil. Stephenson found that the sandy soil had very poor nitrifying power when compared with the humus soil, and from his tables it is evident that in the sandy soils from this sampling, particularly in the case of the protein treatments, there were very few nitrates, while in the humus soil nitrates in large amounts were present. The explanation for the difference between the two soils in the unknown soluble non-protein nitrogen is probably bound up in the difference in their nitrifying power. Nitrification is, of all the phases of biologic activity in soils, perhaps the most sensitive to soil acidity, or conversely the presence of lime is most necessary for its vigorous function. In the production of nitrates the organism takes into its cell nitrogen, no doubt, from the simpler nitrogenous bodies when these are available. In the case under consideration, in the sandy soil there is not a vigorous nitrifying flora and hence in the limed soil there is no great assimilation of nitrogen. In fact there is not as much assimilation as in the unlimed. It will be observed that in the remainder of the sandy soil pots the unlimed soils show not as much excess unknown soluble non-protein nitrogen over their corresponding limed soils as in the case of the humus soil.

#### CONCLUSIONS

1. In order to obtain the maximum soluble non-protein nitrogen from basic soils they should be extracted with 1 per cent hydrochloric acid until the wash water from the extracted soils shows no calcium, or at most a trace of it.
2. It is not necessary at all to extract acid soils with 1 per cent acid in the soluble non-protein nitrogen determination.
3. Devarda alloy in weakly alkaline solution has been found a practicable method for the complete reduction of nitrates in the 1 per cent acid extract of soil.
4. The unknown soluble non-protein nitrogen is usually decreased by lime but there are some striking exceptions to this rule.



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## CAN WE PREDICT PROBABLE FERTILITY FROM SOIL BIOLOGICAL DATA?

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Among the many processes active in soils, whereby both mineral and organic materials are rendered available for absorption by the higher plants, none are more important than the normal functioning of bacteria and fungi. In fact we know that any soil, deprived of its microscopic flora, soon becomes a barren waste incapable of producing normal plants. Bacteria and fungi are very low forms of plant life requiring the same mineral elements for their growth and multiplication as do the higher crop plants with which we are more directly interested. Is it not possible, therefore, that there may exist a relationship or a correlation between certain bacterial activities and the actual crop-producing powers of soils? In an endeavor to answer this question, several investigators during the past few years have attempted to correlate the fertility (or crop-producing power) of soils with their abilities to biologically produce ammonia, nitrates or carbon dioxide, and a few soil scientists have been fairly successful in predicting fertility, or lack of it, from such biological data. Brown (3, 4) at the Iowa Agricultural Experiment Station; Lipman (13) at California; Lyon, Bizzell and Conn (18) at New York; Gainey (8) at Kansas; Given (9) at Pennsylvania; Ashby (1) and Russell and Hutchinson (21), in England; and Vogel (22, 23, 24) and several other German investigators, may be cited as having contributed to our knowledge along these lines of work. That there is often little agreement, either in the data obtained or in the conclusions drawn therefrom by these investigators, is seen by a comparison of their several efforts. This is doubtless due, at least in large part, to the dissimilarity of methods employed. As has been inferred, the attempt has been made in the work above cited to in some degree correlate the yields from various soils with their abilities to biologically transform nitrogen compounds. The organic material used has thus usually remained constant in kind and amount while the soils have varied. Conversely, by allowing the soil to remain constant, it is possible to compare the availabilities of different nitrogenous fertilizers (especially organics) biologically, and at present such methods are largely used, either by determining ammonifiability, as proposed by J. G. Lipman (17) or better, by determining nitrifiability as advanced by Lipman and Burgess (14, 15). Purely chemical methods, as the old alkaline-potassium-permanganate method (2), are no longer con-

sidered reliable for this purpose. Of course, the absolute method of determining fertilizer availability is by carefully controlled vegetation tests, but as these are both costly and time-consuming, and as such experiments have shown the validity of the bacteriological tests above indicated, the latter are now very widely used.

As has been pointed out by the writer and by others, Hawaiian soils are decidedly different, physically, chemically, and biologically, from mainland soils. Many more or less unproductive soils are received at this laboratory from time to time with inquiries as to the causes for conditions noted in the field. Unless a preliminary examination shows, at once, the probable cause of infertility, the soil sample is usually run for available potash and phosphoric acid (sometimes for total phosphoric acid), nitrate nitrogen, total nitrogen, the lime requirement (Veitch method) if acid, and the alkali content is determined if an excess of soluble salts is present. Beside these chemical tests, it is always desirable to learn something as regards the biological properties of the soils submitted. The question thus naturally presents itself, "Which of the several biological processes occurring in soils, i.e., ammonification, nitrification, nitrogen fixation, or carbon-dioxide production, is the best to use as a criterion of fertility in our Hawaiian soils?" In an endeavor to answer this question the following work was planned and carried through.

#### EXPERIMENTAL

About 15 or 20 pounds of surface soil were obtained from each of the following sugar plantations:

Soil no. 2 was probably the most fertile one used, although soils no. 1, 8, 4, 6, and 3 were from very good fields. Soil no. 9 was probably the least fertile, while nos. 5 and 7 were but slightly better. As soon as received at the laboratory, the soils were partially air-dried in the shade and sifted through a 5-mm. mesh sieve, the usual precautions being taken to prevent contamination. The soils were all subjected to the following tests: ammonification, nitrification, nitrogen fixation and total water-soluble nitrogen production. The determinations of the amounts of carbon dioxide formed were not here attempted for it has been recently shown (19) that there is a more or less definite correlation between the amounts of  $\text{CO}_2$  produced by a soil's flora from a given amount of organic material and the quantities of ammonia simultaneously evolved, and further, the accurate determination of  $\text{CO}_2$  as given off from bacterial cultures is not only most difficult, but also the incubation conditions under which the soils must be held during the test are so artificial as to detract somewhat from the value of data so obtained. We also know that a considerable proportion of the  $\text{CO}_2$  evolved from soils is due to mold action (20) and the part played by fungi in soil fertility is at present imperfectly known.

The following fertilizers were used in ascertaining the ammonifying, nitrifying and the total water-soluble nitrogen-producing efficiencies of the

above soils: dried blood (13.5 per cent N), alfalfa meal (3 per cent N), and fish scrap (7.73 per cent N).

Triple portions (50 gm. for the ammonifications and 100 gm. for the nitrifications and total water-soluble nitrogens) of each of the soils were weighed out into sterile glass tumblers and covered with Petri dishes, for each of the three fertilizing materials above given, in so far as the amounts of the soils on hand permitted. As the tables following will show, the samples of soil taken proved to be too small (due to the removal of stones and trash when sifted) always to allow for the use of the three fertilizing materials, hence, in many cases, the fish scrap treatments were omitted.

Three incubation periods were employed for the ammonification, nitrification and total water-soluble nitrogen tests, i.e., 10 days, 20 days and 30 days. At the end of each period one culture of each series was removed and analyzed. The blanks on the untreated soils have in each case been subtracted from the results as tabulated in the tables which follow.

TABLE 1  
*Soils employed*

LABORATORY NUMBER	PLANTATION	*DESCRIPTION OF SOIL
1	Hawaiian Sugar Co.	Red Clay loam; fairly fertile
2	Hawi Mill & P. Co.	Brown silty clay loam; very fertile
3	Laupahoehoe S. Co.	Brown silty clay loam; very good soil
4	Honomu S. Co.	Brown silty clay loam; very good soil.
5	Kipahula S. Co.	Grayish clay loam; poor soil
6	Waipio Substation	Light brown silt loam; fairly fertile
7	Honomu S. Co.	Light brown clay loam; poor; not as good as No. 4
8	H. S. P. A. Exp. Sta., Honolulu	Dark brown clay loam; very good soil
9	Pacific Sugar Mill	Light brown clay loam; poor, acid soil

\* The descriptions of these soils are based upon the crops of sugar cane which they are producing in the field with normal fertilization (1000 to 1500 pounds complete fertilizer per acre).

*The ammonification results.* The usual direct soil culture, or "beaker method," was here employed. To 50 gm. of soil were added 1 gm. of each of the organic materials, irrespective of their nitrogen contents. The incubation temperature was 28°C. As stated above, three different incubation periods were allowed, i.e., 10 days, 20 days and 30 days. At the end of each period one culture using dried blood, one using alfalfa meal and one using fish scrap, were analyzed for ammonia by the usual magnesium oxide distillation method. The results secured at the end of each incubation period and with each fertilizer used are presented in table 2 which follows. In the column "Laboratory Numbers," the numbers refer to the soils employed, and correspond to those given in table 1, while the letters refer to the fertilizing materials used, i.e., A. dried blood; B. alfalfa meal; and C. fish scrap.

The usual incubation period for ammonification studies is from 7 to 10 days and the most common organic material used for this test is finely ground dried blood. For this reason, let us briefly discuss the relative fertilities of the various soils as shown when dried blood was used and the analyses were made at the end of 10 days. Soil no. 2, which is certainly one of the most fertile soils used (if not the very best), heads the list, followed by soils no. 1, 9, 4, 6, 8, 3, 7, and 5. Soil no. 5 is certainly one of the poorest ones and it shows the lowest ability to ammonify, but, on the other hand, soil no. 9 is also very poor while it is third on the list, lacking but a few tenths of a per cent of being next to the best soil noted. Soil no. 3, while it stands near the

TABLE 2  
*The ammonification results*

LABORATORY NUMBER	10-DAY INCUBATION PERIOD		20-DAY INCUBATION PERIOD		30-DAY INCUBATION PERIOD	
	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified	Mgm. added nitrogen ammonified	Per cent added nitrogen ammonified
1A	76.4	56.6	50.4	37.3	35.6	26.4
1B	6.7	22.3	3.6	12.0	2.8	9.3
1C	41.4	53.8			24.9	32.3
2A	100.2	74.2	65.5	48.5	59.9	44.4
2B	14.8	49.3	12.0	40.0	5.6	18.7
3A	57.1	42.3	63.0	46.7	46.5	34.4
3B	12.9	43.0			4.8	16.0
4A	74.5	55.2	57.4	42.5	40.0	29.6
4B	9.8	32.7	11.5	38.3	2.5	8.3
5A	26.6	19.7	29.7	22.0	25.8	19.1
5B	1.4	4.7	1.1	3.6	2.2	7.3
5C	16.0	20.8	1.9	2.5	4.4	5.7
6A	68.6	50.8	70.0	51.8	46.5	34.4
6B	11.2	37.3			5.3	17.7
7A	47.6	35.3	61.0	45.2	45.9	34.0
8A	57.7	42.7	15.4	11.4	31.9	23.6
8B	9.5	31.7			1.7	5.6
9A	75.9	56.2	74.2	55.0	80.1	59.3
9B	14.0	46.7	20.7	69.0	21.6	72.0

lower end of the list, is nevertheless a very good producing soil. Many of the intermediate soils are, however, quite close together in the percentages of added nitrogen which they have been able to ammonify.

Where finely-ground alfalfa meal (a vegetable protein material) was used as the source of nitrogen, certain changes in the relative standing of the soils are seen, although no. 2 still heads the list and no. 5 concludes it as before. Soil no. 1, which before was next to no. 2, is now next to the last, while no. 3, which with dried blood did poorly, ranks third. Soil no. 9 holds second place while it was a close third before, notwithstanding the fact that it is a very poor, acid upland soil. It may be said here that there was considerable mold

growth noticed in the no. 9 cultures (especially when dried blood was used), hence probably much of the ammonia here produced was of fungal origin. With alfalfa meal the soils rank as follows: 2, 9, 3, 6, 4, 8, 1, and 5.

Fish scrap was used only in two instances, but here it shows soil no. 1 to be much better than soil no. 5, which is in accordance with fact and with other tests.

As will be seen, in almost all cases the amounts of ammonia found at the end of the 20- and 30-day incubation periods are much lower than those found at the end of 10 days. As the former periods are too long for maximum ammonification, the data presented are probably of little value, although it will be noticed that there is but little change in the relative positions of the different soils throughout the 30 days.

Purified egg albumen, cottonseed meal and tankages, besides the materials herein employed, have in the past been compared by the author with dried blood as to ammonification in Hawaiian soils. The last, however, has been found to give results slightly more in accord with field conditions, and it is now used almost entirely in work of this character.

From the data presented it is evident that ammonification is not, as a rule, suitable in differentiating between good and poor Hawaiian soils, although it may show differences between *very poor* and *very good* soils. Similar conclusions were reached by Kelley (12), also working with Hawaiian soils. He found that, as a rule, good and poor soils alike supported vigorous ammonification, while nitrification was a variable quantity depending more or less upon aeration. This work also bears out the conclusion of Gainey (8), working on Kansas soils, who says, "A study of the data here presented, secured from widely different localities, soil types, and variations in productivity, convinces anyone of the absence of any correlation between yield and ammonia nitrogen content." On the other hand, Brown (3, 4) secures ammonification results in complete agreement with crop yields. He writes, "It would seem, therefore, that there must be some close relationship between the ammonifying power of soils and their crop production."

*The nitrification results.* Kelley (12) has recently shown that, for nitrification tests, smaller quantities of nitrogen carrying materials are to be preferred to the older methods where the equivalent of from 1 to 2 per cent of dried blood was used. A better differentiation between both fertilizers and soils is possible where amounts of organic materials somewhat in accord with actual field practice are employed. For these reasons 30 mgm. of nitrogen from each of the three organics were employed per culture in these nitrification tests. The actual amounts of each fertilizer required to furnish 30 mgm. of nitrogen were:

	gm.
A. Dried blood (13.5 per cent N).....	0.222
B. Alfalfa meal (3.0 per cent N).....	1.000
C. Fish scrap (7.73 per cent N).....	0.388

The beaker method, employing 100 gm. of soil per culture was used throughout. The incubation temperature was 28°C. At the end of each of the 3 incubation periods (10, 20 and 30 days) one culture from each series was removed and analyzed for nitrates by the modified phenol-disulphonic-acid method (16). All of the results obtained appear below in table 3.

It is usually found that the optimum incubation period for nitrification in soil cultures under laboratory conditions is from 4 to 5 weeks. The three periods, 10, 20 and 30 days, were here chosen in order that the rates of nitrification, as well as the absolute amounts of nitrate formed during one month, might be ascertained. With the exception of the poor soils, no. 5 and 9, it

TABLE 3  
*The nitrification results*

LABORATORY NUMBER	10-DAY INCUBATION PERIOD		20-DAY INCUBATION PERIOD		30-DAY INCUBATION PERIOD	
	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified	Mgm. added nitrogen nitrified	Per cent added nitrogen nitrified
1A	4.7	15.7	15.2	50.6	15.2	50.6
1B	3.8	12.7	9.0	30.0	9.0	30.0
1C	4.4	14.7			12.0	40.0
2A	2.5	8.3	13.6	45.3	20.8	69.3
2B	1.8	6.0	8.0	26.7	15.2	50.6
3A	3.2	10.7	12.0	40.0	20.0	66.7
3B	1.8	6.0			12.0	40.0
4A	4.7	15.7	14.0	46.7	18.0	60.0
4B	4.7	15.7	11.0	36.7	12.8	42.7
5A	6.0	20.0	6.0	20.0	4.0	13.3
5B	3.8	12.7	5.6	18.7	7.2	24.0
5C	9.0	30.0	9.4	31.3	5.0	16.7
6A	1.8	6.0	9.0	30.0	17.2	57.3
6B	0.9	3.0			9.6	32.0
7A	4.0	13.3	12.0	40.0	13.6	45.3
8A	4.5	15.0	16.0	53.3	16.8	56.0
8B	6.0	20.0			10.0	33.3
9A	7.0	23.3	4.0	13.3	4.0	13.3
9B	4.4	14.7	5.6	18.7	4.5	15.0

will be noted that there was a steadily increased production of nitrate nitrogen from the tenth to the thirtieth day in almost all cases. Especially is this noticed with the best soils under examination, i.e., no. 2, 3, 4 and 8. With soils 5 and 9 there was a decrease after the first 10-day period in many cases. This was possibly due to denitrification in some cases while in others the rapid evolution of ammonia occurring at first might have had a tendency to correct existing acidity, thus initially rendering conditions more nearly suitable for nitrification.

Let us now arrange the soils in their order of nitrate production (as shown at the end of the 30-day incubation period where dried blood was used).

Beginning with the best, the soils fall in the following sequence: no. 2, 3, 4, 6, 8, 1, and 7, while 5 and 9 happen to be the same, and are the lowest. The first four or five soils thus listed are all of high productivity, and, as table 3 shows, differ but little in the large percentages of added nitrogen nitrified, while no. 5, 9 and 7 are the lowest nitrifiers, and we know them to be the poorest soils.

Where alfalfa meal was used in place of dried blood (but supplying exactly the same amount of nitrogen, 30 mgm.), the soils group themselves in the following order: no. 2, 7, 4, 3, 8, 6, 1, 5, and 9. Soil no. 2 still heads the list, while no. 5 and 9 remain the poorest. The only change of note is in the case of soil no. 7 which here occupies second place where with dried blood, it came later. Soil no. 7 is not a productive soil and why here it should occupy a post so near the front cannot be explained unless it was accidentally contaminated, or unless the causes of its infertility are other than low nitrifying efficiency. That the latter is in part true is known to the writer who made a careful study of these two Honomu soils (no. 4 and 7) over a year ago and submitted his findings at that time to the manager of the Honomu Sugar Company. The results of the chemical work performed upon these two soils at that time were as follows:

SOIL	CITRATE-SOLUBLE			TOTAL NITROGEN	LIME REQUIREMENT TONS PER ACRE-FOOT (VEITCH)
	CaO	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1*	0.083	0.0076	0.0118	0.53	7.5
2	0.054	0.0046	0.0087	0.34	12.2

\* No. 1 and 2 are those numbered no. 4 and 7 elsewhere in this present paper.

From this work we see that soil no. 7 is in all respects a poorer soil than no. 4. These two samples were taken by our assistant agriculturist, Mr. W. P. Alexander, on July 9, 1917, and were described by him as follows:

Sample 1. Below the ditch is an area which has grown very good cane. It is in a pocket, i.e., there is drainage from 2 slopes. It was the former site of a Hawaiian kuleana. The soil is deep. The sample was taken from 3 separate holes dug 1 foot deep. A bag was laid in the bottom of the hole and a slice of the side of the hole taken with a spade. This soil was collected on the bag. The 3 separate "slices" of soil were mixed, and constitute the sample.

Sample 2. Above the ditch is an area which produced poor cane. It has grown vegetables in the past. The soil is shallow, as it is more like a ridge where this area is located. The sample was secured from 3 separate holes, as above described.

From an examination of the data as given in table 3 it is evident that the nitrification figures, where dried blood is used and an incubation period of 30 days is employed, are a very good comparative measure of the crop-producing powers of the various soils under discussion. It may be stated here that the writer has examined a large number of Island soils for nitrification efficiency, using both dried blood and ammonium sulfate as sources



of nitrogen, and almost invariably the figures secured are in accord with the known productivity of the soils so tested. Dried blood usually gives more comprehensive results than ammonium sulfate, or, in fact, than any of the other materials tried.

The findings of the writer, as here stated, are in accord with results secured by several investigators on the mainland. Gainey (8), working with Kansas soils, writes:

As pointed out before there is evidently a correlation between nitrification and yield but not between any other two factors under consideration.

Also,

We believe, therefore, that while there is usually a correlation between nitrifying power and productivity, it does not imply that the processes of nitrification are responsible for yield or that yields on non-fertile soils are limited by the process of nitrification. As to whether high nitrifying powers are the result of high fertility or that both are the result of common factors, there are very few data to indicate. Since it is not impossible that both factors depend upon available plant-food, we would call attention to the work of Lipman (13) who has detected a relation between yields and certain available inorganic elements, also to the work of Fraps (7) who has detected a relation between nitrogen content of soils and their nitrifying powers.

I may state here that there is, in Hawaiian soils, little correlation between nitrogen content and nitrification, doubtless due, in large part, to varying degrees of soil acidity.

Brown (3, 4) of Iowa, Given (9) of Pennsylvania, and Lipman (13) of California, have all been able to show a direct relationship between nitrification and crop production.

*Total nitrogen rendered water-soluble.* These tests were set up in exactly the same way as were the nitrification cultures—100 gm. of soil in tumblers plus 30 mgm. of the different forms of organic nitrogen. The temperature and periods of incubation were also the same. At the end of each period one culture of each series was removed and, with 400 cc. of nitrogen-free distilled water, washed into a quart Mason jar. The jars were shaken for one hour in a shaking machine then allowed to stand over night when practically all of the soil had settled out leaving the supernatant liquid slightly cloudy. The latter was then carefully and completely siphoned off leaving the moist soil in the bottoms of the jars. The liquids were clarified by passing them through Pasteur-Chamberland clay filters under pressure. The first 75 cc. was discarded. Another 400 cc. of distilled water was now added to the soils in the jars and the same process repeated. Exactly 200 cc. of each first clear filtrate was mixed with exactly 200 cc. of the second extraction of the same soil (which represented 50 gm. of the soil culture). Ten cc. of concentrated sulfuric acid was then added to each solution and the whole reduced to a bulk of about 100 cc. on the water bath. When cool, each solution was transferred to a Kjeldahl flask, about 8 gm. of iron (reduced by hydrogen) was added,

and the whole allowed to stand over night to reduce the nitrates present (Ulsch's (2) modified method). Most of the water was then carefully boiled off, 30 cc. more of strong sulfuric acid was added, together with about 10 gm. of salt mixture (10 parts  $K_2SO_4$ , 1 part  $FeSO_4$  and  $\frac{1}{2}$  part  $CuSO_4$ ) (10) and the whole digested two hours to obtain any soluble organic nitrogen which might have been present. As color was noted in several of the original solutions, it indicated the presence of soluble organic compounds. The digestions were then neutralized and distilled as in the usual Kjeldahl determination for total nitrogen.

TABLE 4  
*The total nitrogen rendered water-soluble*

SOIL NUMBER	10-DAY INCUBATION PERIOD				20-DAY INCUBATION PERIOD				30-DAY INCUBATION PERIOD			
	Mgm. nitrogen from H <sub>2</sub> O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen re-covered	Per cent nitrogen rendered H <sub>2</sub> O soluble	Mgm. nitrogen from H <sub>2</sub> O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen re-covered	Per cent nitrogen rendered H <sub>2</sub> O soluble	Mgm. nitrogen from H <sub>2</sub> O solutions	Mgm. nitrogen from soils	Total mgm. nitrogen re-covered	Per cent nitrogen rendered H <sub>2</sub> O soluble
1A	11.9	8.4	20.3	67.7	14.7	3.6	18.3	61.0	14.0	2.5	16.5	55.0
1B	8.4	2.2	10.6	35.3	11.2	3.4	14.6	48.7	10.5	2.5	13.0	43.3
1C	11.9	6.9	18.8	62.7					12.6	2.2	14.8	49.3
2A	9.1	17.9	27.0	90.0	15.4	14.0	29.4	98.0	19.8	1.4	21.2	70.7
2B	7.7	13.4	21.1	70.3	12.6	9.5	22.1	73.7	15.4	3.3	18.7	62.3
3A	11.9	9.8	21.7	72.3	16.8	7.6	24.4	81.3	16.8	2.8	19.6	65.3
3B	8.4	8.4	16.8	56.0					13.3	4.7	18.0	60.0
4A	12.6	7.5	20.1	67.0	17.5	6.4	23.9	79.7	14.7	2.5	17.2	57.3
4B	9.8	3.9	13.7	45.7	14.0	4.5	18.5	61.7	12.6	3.6	16.2	54.0
5A	7.7	0.5	8.2	27.3	8.4	1.9	10.3	34.3	4.2	1.4	5.6	18.7
5B	6.3	0.5	6.8	22.7	9.1	1.6	10.7	35.7	10.5	2.5	13.0	43.3
5C	9.1	1.2	10.3	34.3	11.9	1.1	13.0	43.3	9.1	1.1	10.2	34.0
6A	9.8	14.5	24.3	81.0	16.1	10.9	27.0	90.0	16.5	1.9	18.4	61.3
6B	7.7	6.4	14.1	47.0					14.0	3.6	17.6	58.7
7A	9.1	6.1	15.2	50.7	14.7	7.6	22.3	74.3	10.5	1.9	12.4	41.3
8A	14.7	15.0	29.7	99.0	16.1*	6.7	22.8	76.0	18.2	3.3	21.5	71.7
8B	9.1	5.6	14.7	49.0					13.3	3.6	16.9	56.3
9A	11.2	10.9	22.1	73.7	16.1	15.0	31.1	103.7	18.2	12.0	30.2	100.7
9B	12.6	10.0	22.6	75.3	17.5	12.6	30.1	100.3	14.0	16.0	30.0	100.0

That certain soluble forms of nitrogen (ammonium salts, amido-acids, amines, etc.) are quite strongly adsorbed by soils and are retained at least in part against washing with pure water, is an established fact. Hence the moist soils, from whence the supernatant liquids had been withdrawn, were transferred to copper flasks and distilled with magnesium oxide. The sum of these two portions, minus the blanks on the soils examined, represents probably very closely the total amounts of water-soluble nitrogen formed by the soil flora from the added insoluble organic materials.

The results as secured by this method appear in table 4. The first columns under each incubation period show the mgm. of nitrogen (blanks subtracted)

which were secured in the water extracts; the second columns, the mgm. found in the residual soils after the extractions (blanks subtracted); the third columns, the sum of these two; and in the last columns under each time period appear the percentages of added organic nitrogen rendered soluble.

A perusal of table 4 shows that the highest percentages of organic nitrogen rendered water-soluble were secured at the end of the 20-day incubation period. Under the conditions of this test we measure both the ammonia nitrogen and the nitrate nitrogen produced, as well as the simpler protein compounds and decomposition products which have been rendered water-soluble by the soil bacteria and fungi. That there is a gradual increase in nitrate nitrogen from the first period to the last, and that the reverse is shown for ammonia production, is brought out by comparing the nitrogen in the water-soluble portion (which doubtless contains practically all of the nitrate) with that recovered by distilling the residual soils with magnesia (which, due to adsorption, probably carries a large part of the ammonia). This fact is possibly better shown by comparing the "mgm. of added nitrogen nitrified" in table 3 with the "total mgm. of nitrogen recovered" from the soils as shown in table 4, for both sets of cultures were similar in the essential details, and should be fairly comparable.

It is to be regretted that cultures were not analyzed at the 20-day period for soils 1C, 3B, 6B and 8B. This was due to lack of soil. However, where dried blood was employed as the source of nitrogen (the "A" cultures), a complete series at the 20-day incubation period is before us for discussion. Let us consider the relative percentages of added blood nitrogen rendered soluble by the several soils. Soil no. 9 heads the list with all of the added nitrogen rendered soluble. It will be recalled that this is one of the poorest soils under discussion, also that its ammonification coefficient was similarly high. The table shows us that a large percentage of this soluble nitrogen is here present as ammonia. The soils occur in the following order, depending on their abilities to render blood nitrogen soluble: no. 9, 2, 6, 3, 4, 8, 7, 1, and 5. It is evident that this order does not correspond with the known fertility of these soils although, with the exception of no. 9 and 1, there is a general tendency for the better soils to appear first.

In so far as data are available for the alfalfa meal series (the "B" cultures), the soils appear in exactly the same order as in the blood series, i.e., no. 9, 2, 4, 1, and 5.

From a careful study of table 4 it is evident that the amount of organic nitrogen rendered water-soluble by a soil's flora is hardly a criterion whereby we may judge of its inherent capacity to produce crops, or whereby comparative fertilities may be differentiated, except in a very general way.

*Nitrogen fixation.* Due to lack of soil, it was impossible to determine the nitrogen-fixing powers of these soils by the beaker, or soil culture, method. Mannite solutions (5) were therefore made up carrying 1 gm. of mannite per 100 cc. Wide-mouthed Erlenmeyer flasks of 500 cc. capacity, each re-

ceiving 100 cc. of solution, were sterilized and, when cool, inoculated with 1 gm. each of the soils under examination. These cultures were incubated 3 weeks at 28°C., after which total nitrogen determinations were made. These figures (minus the amount of nitrogen introduced with each gm. of soil used as an inoculum) together with the appearance of the cultures, appear in table 5.

Let us briefly consider the data as here given. The three poorest soils certainly fixed the least nitrogen, while soils no. 2 and 8, which are certainly among the best soils under discussion, fix the most. *Azotobacter* forms were entirely absent from cultures 5 and 9, while their presence in 7 was doubtful. All of the other soils, except possibly no. 1, gave cultures showing good *Azotobacter* surface membranes. These nine soils fall naturally into three classes depending upon their nitrogen-fixing abilities. The first or best is comprised of soils 2 and 8 which fix about 11 mgm. of nitrogen each where 1 gm. of mannite is supplied as the source of energy. To the second class belong

TABLE 5  
*The nitrogen-fixation results*

SOIL NUMBER	MGM. NITROGEN FIXED PER CULTURE (PER 1 GM. MANNITE)	PRESENCE OF AZOTOBACTER
1	5.60	?
2	11.20	+
3	6.44	+
4	7.00	+
5	2.80	—
6	7.28	+
7	3.92	?
8	10.64	+
9	3.60	—

soils 6, 4, 3, and 1. These are able to fix about 6 or 7 mgm. under the same conditions. The third or poorest class composed of soils 5, 7, and 9, are able to secure less than 4 mgm. of atmospheric nitrogen under the conditions imposed.

Arranging the soils in the order of their abilities to fix free nitrogen in mannite solution cultures we have: no. 2, 8, 6, 4, 3, 1, 7, 9, and 5. Where nitrate production (nitrification) was the measure of comparative fertility employed, it will be recalled that the following order was shown: no. 2, 3, 4, 6, 8, 1, 7, 5, and 9, which, considering the slight differences obtaining in both cases between the results secured with soils 6, 4, 3, and 1, is certainly a remarkably close agreement. Brown (3) of Iowa has also shown a very complete agreement between nitrogen fixation and nitrification.

#### SUMMARY

The object of this work, as stated in the title, was to ascertain whether or not it is possible to predict, with any degree of accuracy, the crop-producing

powers of Hawaiian soils, or rather their relative crop-producing powers, from microbiological data, i.e., ammonification, nitrification, nitrogen fixation, etc., and if so, which is the best criterion to use in routine, comparative tests.

Nine Hawaiian surface soils, all of which had been under sugar cane cultivation for many years, were chosen for this investigation. Two or three of them were of exceptional fertility, three were capable of yielding good average crops, while three produced poor crops of cane even after fair fertilizer applications.

These soils were brought to the laboratory, carefully sifted through a 5-mm. sieve, after being air-dried in the shade, and the following tests made upon them: ammonification, nitrification, total supplied organic nitrogen rendered water-soluble, and nitrogen fixation. Three incubation periods were used, viz., 10, 20, and 30 days, while three different organic forms of nitrogen (dried blood, fine alfalfa meal and fish scrap) were employed. The ordinary precautions against contamination were taken.

The methods used, together with the results obtained, are discussed.

The following conclusions seemed justified:

1. Ammonification tests are not suitable to differentiate between the fertilities of average Hawaiian soils, although they will often show differences between *very poor* and *very good* soils.

2. The abilities of soils to render organic nitrogen (of blood or alfalfa) water-soluble are of no value as measures of their crop-producing powers.

3. Nitrification (soil culture method) is by far the most accurate biological soil test yet perfected for predicting probable fertility. In fact, it is probably the best single test of any description yet developed for ascertaining the comparative crop-producing powers of arable soils. At least, this holds for Hawaiian soils, and it may be added that the writer has tested scores of Island soils by the method for nitrification as given herein, and in only a very few instances have the nitrification coefficients been at variance with the known fertility of the fields from whence the samples were drawn. Of course there are exceptions, and it must not be inferred that nitrification tests are able to take the place of carefully conducted chemical or vegetation experiments. Active nitrification may not be the *cause* of high fertility, yet those conditions which tend to promote rapid nitrification are very evidently identical with those which tend to give us enhanced crop yields. Furthermore, although nitrification tests may be a means of differentiating between good and poor soils, they do not tell us the *causes* of the differences noted, nor do they show us exactly how to *improve conditions* in soils of low productivity. Chemical, physical and plant physiological experiments alone are able to give us this information. A discussion of these phases of soil work falls beyond the limits of this paper.

4. There was a remarkable correlation between the amounts of nitrogen fixed in mannite solution cultures and the known fertilities of the soils studied. There was little difference in the comparative rating of the soils, depending

upon whether nitrification or nitrogen fixation tests were used as the criteria of fertility. *Azotobacter* species are seldom present in Hawaiian soils of low productivity; the more fertile soils, however, carry several species (6), and in so far as fixation is concerned, compare favorably with mainland soils of similar crop-producing powers.

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## TOXICITY OF "ALKALI" SALTS<sup>1</sup>

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The detrimental effect of the accumulation in soils of the so-called alkali salts on crop growth has long been recognized and the importance of its control appreciated. Considerable work has been done to determine the concentration at which the different salts become toxic to plant growth. The more recent work, notably that of C. B. Lipman, has pointed out the importance of the effect of various alkali salts on bacterial activities. Most of the experiments up to the present time have been carried on with single alkali salts rather than with combinations of salts as they exist under field conditions. Some of the studies have included certain arbitrary combinations. The purpose of the work recorded in the following pages is to attempt to throw more light on this problem under conditions existing in Oregon.

### HISTORICAL

As early as 1884 Warington (19) showed that the presence of 0.032 per cent of sodium bi-carbonate retarded nitrification and that in the presence of 0.096 per cent of this salt, nitrification was very slight. Schloesing (18) added various salts to soils in quantities not exceeding 485 part per million, but found no apparent effect on nitrification. Deherain found that sodium chloride became toxic at a concentration of 0.1 per cent and when higher concentrations were used, nitrification ceased. He also found that sodium nitrate may stop nitrification for a time but at higher concentrations stimulates it.

J. G. Lipman (14) showed that sodium chloride was injurious to nitrifying organisms; and that (17) a distinct decrease was produced as the application of sodium chloride was increased. When 0.1 per cent was added, nitrification was greatly diminished. J. G. Lipman and Brown (15) found that nitrifications were accelerated by sodium nitrate. In a later work they found that sodium nitrate increased the accumulation of nitrates in soils but that a

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certain periodicity in the accumulation existed. This would account for the different results obtained by different investigators. McBeth and Wright (16) found that carbonates, chlorides, and sulfates inhibited nitrification and that the former were more injurious than the latter.

In a recent work Brown and Hitchcock (1) show that nitrification in a normal soil is stimulated by small amounts of sodium chloride, sodium sulfate, magnesium sulfate and by large amounts of calcium carbonate. Their toxic points for a normal soil under laboratory conditions are sodium chloride 0.02 per cent, sodium sulfate 2 per cent. and calcium carbonate between  $1\frac{1}{2}$  and 6 per cent. Nitrification in an alkali soil was increased by small amounts of sodium bi-carbonate, sodium carbonate and calcium carbonate. Sodium carbonate and sodium bi-carbonate became toxic at 0.3 per cent and calcium carbonate at 0.6 per cent. When crops were grown on the soil, a similar effect was noted on the crop as in the case of nitrification.

Recent investigation by Kelley (5) on nitrification in semi-arid soils show that 0.03 per cent of sodium carbonate is distinctly toxic to the nitrification of 1 per cent dried blood, but that concentrations as high as 4 per cent had no effect on the nitrification of 1 per cent dried blood. When 0.15 per cent of ammonium sulfate was used, 0.1 per cent of sodium carbonate became toxic to nitrification, but this same amount of sodium carbonate stimulated nitrification when 0.0635 per cent of ammonium sulfate was used. Similar results were obtained when sodium sulfate was used in place of the carbonate. According to Engberding (2) ammonium sulfate, sodium nitrate, potassium nitrate and caustic lime all increased the bacterial content of soil, but on the other hand decreased the nitrogen fixing power. Lipman, C. B. and Sharp (10) found that 0.5 per cent to 0.6 per cent of sodium chloride was toxic to nitrogen fixation, and that 1.25 per cent of sodium sulfate and 0.4 to 0.5 per cent of sodium carbonate was also toxic. Sodium chloride was the only one that acted as a stimulant at any concentration.

Work by Peck (17) showed that sodium nitrate depressed ammonification while the sulfate, phosphate and carbonate of calcium caused an increase. J. G. Lipman's (15) work showed an increase in ammonification due to sodium nitrate, while work done by C. B. Lipman (8) showed that ammonification was inhibited by the chloride, sulfate and carbonate of sodium. Sodium chloride became toxic at a concentration between 0.1 and 0.2 per cent, Sodium sulfate at 0.4 per cent and sodium carbonate at 2 per cent. A stimulating effect was noticed in some cases for sodium carbonate but none in the case of the sulfate and chloride. In a later work (9) he found the following toxic points: for sodium chloride less than 0.1 per cent, for sodium sulfate 0.35 per cent and for sodium carbonate 0.025 per cent. All except the carbonate acted as stimulants.

Recently C. B. Lipman (11) has demonstrated that there exists as measured by ammonification a true antagonism between sodium chloride and sodium sulfate and between sodium sulfate and sodium carbonate. In a pre-

vious work (7) he showed that the chlorides of calcium, magnesium, potassium and sodium were toxic in the order named. He also found that no antagonism existed between the calcium ion and the magnesium ion or between the calcium and sodium ion. Marked antagonism existed between calcium and potassium, magnesium and sodium and between potassium and sodium.

Greaves (3) states that the salts of sodium chloride, calcium chloride, sodium sulfate and calcium nitrate are very toxic to ammonifying organisms and their presence to any extent in the soil will greatly reduce ammonification. The compounds that are the most active stimulants for higher plants are also the most active for ammonifying organisms.

Harris (4) has carried on quite extensive work to determine the toxicity of alkali salts both alone and in combination to plant growth. In his work he concludes with the following summary:

Land containing more than the following percentages of soluble salts is probably not suited for ordinary crops without reclamation: in loam soils, chlorides 0.3 per cent, nitrates 0.4 per cent, carbonates 0.5 per cent and sulfates 1 per cent. In coarse sandy soil, chlorides 0.2 per cent, nitrates 0.3 per cent, carbonates 0.3 per cent and sulfates 0.6 per cent

#### EXPERIMENTAL

The work carried on was divided in three phases, namely:

(a) The determination of the toxic points of sodium chloride, sodium nitrate, sodium carbonate and sodium sulfate on peas and wheat.

(b) The determination of the toxic point for peas and wheat when a combination of alkali salts were used as found by analysis, in field soils.

(c) The determination of the effect of sodium chloride, sodium nitrate, sodium carbonate and sodium sulfate upon ammonification, nitrification and nitrogen fixation.

The soil used was obtained from the Eastern Oregon Branch Experiment Station of Moro, Sherman County, Oregon. This soil is a heavy silt loam soil and is quite extensive in area throughout the Columbia Basin. Under normal conditions it is quite productive and when properly handled produces large crops of small grains. Under dry farming conditions alkali spots are gradually beginning to develop, while under irrigated conditions, due probably to over-irrigation, alkali soon appears.

The alkali salts present in six different soils were determined by chemical analyses. The soils were selected so that one of the sodium salts predominated in each. The analyses are shown in table 2.

In the greenhouse tests, 250 gm. of soil was placed in tumblers and brought up to optimum moisture content. The different percentages of alkali salts were added and after two weeks the seed was planted. The soils were kept at the optimum moisture content throughout the experiment. Five weeks after germination took place the plants were harvested and the dry weight

obtained. The soils used for the greenhouse test were the Eastern Oregon Silt loam and the six soils of different combinations for the series of salt and and salts in combination, respectively.

TABLE 1  
*Chemical and mechanical composition of the soil*

CHEMICAL ANALYSIS		MECHANICAL ANALYSIS	
	<i>per cent</i>		<i>per cent</i>
Silica (SiO <sub>2</sub> ).....	53.04	Fine gravel.....	0.65
Potash (K <sub>2</sub> O).....	1.18	Coarse sand.....	1.72
Soda (Na <sub>2</sub> O).....	2.06	Medium sand.....	1.50
Lime (CaO).....	7.32	Fine sand.....	10.00
Magnesia (MgO).....	3.15	Very fine sand.....	11.10
Ferric oxide (Fe <sub>2</sub> O <sub>3</sub> ).....	10.40	Silt.....	69.60
Alumina (Al <sub>2</sub> O <sub>3</sub> ).....	15.92	Clay.....	11.27
Phosphorus pentoxide (P <sub>2</sub> O <sub>5</sub> )...	0.28		
Volatile matter.....	6.56		
Humus.....	1.95		

TABLE 2  
*Alkali salts present in soils*

	1	2	3	4	5	6
	<i>per cent</i>	<i>per cent.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
KCl.....	4.00					6.27
Na <sub>2</sub> SO <sub>4</sub> .....		4.67	93.40	58.50		
NaNO <sub>3</sub> .....		12.96				87.14
Na <sub>2</sub> CO <sub>3</sub> .....		75.95		22.9	2.45	4.03
NaCl.....	81.15	1.46	6.60	18.6		2.56
Na <sub>2</sub> PO <sub>4</sub> .....		4.94				
CaCl <sub>2</sub> .....	0.25					
MgCl <sub>2</sub> .....	7.71					
CaSO <sub>4</sub> .....	6.61					
NaHCO <sub>3</sub> .....	0.28				4.05	
KHCO <sub>3</sub> .....					88.26	
CaCO <sub>3</sub> .....					1.76	
					3.48	

In the bacteriological tests, 100 gm. of soil of the Eastern Oregon Silt loam were used. The soil was placed in tumblers and the different salts were added. The soil then was brought to optimum moisture content and an infusion of fresh soil was added to each to insure vigorous bacterial action. The soil was incubated for four weeks at room temperature, the moisture content being kept constant by the addition of sterile distilled water.

*Series 1. The effect of NaCl on nitrogen fixation, nitrification and ammonification*

Each series was run for nitrogen fixation, nitrification and ammonification and differ only from one another in the percentage of the salt used. All work was done in duplicate and the results are expressed in milligrams per million parts of soil. The arrangement and results of Series 1 are shown in table 3.

TABLE 3  
*Effect of NaCl on nitrogen fixation, nitrification and ammonification*

NUMBER	NaCl	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	NO <sub>3</sub>	Average	NH <sub>3</sub>	Average
	<i>per cent</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>
1	None	341.0		50.8		10.06	
2	None	341.0	341.0	89.2	70.0	9.90	9.9
3	0.001	1,262.0		57.0		7.00	
4	0.001	1,195.0	1228.5	80.9	68.9	6.80	10.2
5	0.003	1,333.0		80.9		10.03	
6	0.003	1,885.0	1609.0	60.4	70.2	10.80	10.5
7	0.005	1,701.0		63.0		7.50	
8	0.005	1,388.0	1544.5	64.0	63.5	6.60	7.0
9	0.010	322.0		69.0		4.80	
10	0.010	101.0	211.5	58.0	63.5	5.99	4.9
11	0.050	68.0		50.0		5.80	
12	0.050	71.0	69.5	37.7	43.8	6.50	6.1
13	0.100	76.0		42.0		5.90	
14	0.100	94.4	85.2	34.8	38.4	6.80	6.3
15	0.200	35.8		44.0		9.00	
16	0.200	7.2	21.5	44.0	44.0	8.40	8.7
17	0.400	14.0		30.1		7.40	
18	0.400	7.2	10.6	35.0	32.5	7.30	7.3

According to these results NaCl in small amounts acted as a stimulant for nitrogen fixation. The greatest amount of nitrogen was fixed at a concentration of 0.003 per cent. At 0.01 per cent a distinct decrease occurred which continued as the concentration was increased. No marked increase in nitrification was shown in any case and at a concentration as low as 0.05 per cent a decrease occurred as compared with the check. Ammonification was not stimulated to any extent by any of the concentrations. A slight increase was shown at 0.003 per cent while all others were lower than the check. The points of toxicity were 0.005 per cent for ammonification and nitrification and 0.01 per cent for nitrogen fixation.

*Series 2. The effect of  $\text{Na}_2\text{CO}_3$  on nitrogen fixation, nitrification and ammonification*

This series was arranged and carried on similar to Series 1, the only difference being in concentration of the salt used. The concentrations used were higher than in the case of NaCl.

TABLE 4

*Effect of  $\text{Na}_2\text{CO}_3$  on nitrogen fixation, nitrification and ammonification*

NUMBER	$\text{Na}_2\text{CO}_3$	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	$\text{NO}_2$	Average	$\text{NH}_3$	Average
	<i>per cent</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>
1	None	341		50.8		10.05	
2	None	341	341.0	89.2	70.0	9.9	9.90
3	0.01	341		102.1		17.0	
4	0.01	341	341.0	294.0	198.0	16.6	16.00
5	0.03	341		55.0		21.1	
6	0.03	341	341.0	117.4	86.0	7.7	14.40
7	0.05	342		89.0		15.9	
8	0.05	343	342.0	106.2	97.5	7.4	11.60
9	0.10	205		127.0		13.1	
10	0.10	205	205.0	152.0	139.5	13.1	13.10
11	0.20	137		33.4		10.5	
12	0.20	138	137.5	174.0	103.7	10.8	10.60
13	0.50	138		139.0		11.0	
14	0.50	137	137.5	139.0	139.0	13.2	12.10
15	0.80	68		68.8		10.5	
16	0.80	68	68.0	67.0	67.9	10.8	10.60
17	1.00	27		57.6		9.7	
18	1.00	14	20.5	57.0	57.3	10.0	9.80

At the concentrations used in this work  $\text{Na}_2\text{CO}_3$  showed no stimulating effect on nitrogen fixation. Perhaps, if concentrations as low as those used in the NaCl series, had been used, a stimulating effect might have occurred. This of course would then be at a concentration less than 0.01 per cent. Nitrification was increased by concentration up to 0.5 per cent. Above this it was inhibited when compared with the check. The sodium carbonate increased ammonification at concentration up to as high as 0.8 per cent. The greatest stimulation took place at 0.01 per cent and as the concentration was increased there was a gradual decrease in the ammonification. The toxic points of  $\text{Na}_2\text{CO}_3$  were 0.02, 0.08 and 0.01 per cent for ammonification, nitrification and nitrogen fixation, respectively.

*Series 3. The effect of  $\text{NaNO}_3$  on nitrogen fixation, nitrification, and ammonification*

This series deals with the effect of sodium nitrate on bacterial activities. The results on the amounts of nitrogen fixed in the case of nitrogen fixation are the amounts above what was added to the soil by the addition of the  $\text{NaNO}_3$ . The same is true for the results given in the case of the nitrates in nitrification.

TABLE 5

*Effect of  $\text{NaNO}_3$  on nitrogen fixation, nitrification and ammonification*

NUMBER	Na $\text{NO}_3$	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	$\text{NO}_3$	Average	$\text{NH}_3$	Average
	<i>per cent</i>	<i>mgm. p. m.</i>	<i>mgm p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>
1	None	341.0		50.8		10.06	
2	None	341.0	341.0	69.2	70	9.90	9.9
3	0.01	359.0		69.0		8.60	
4	0.01	72.0	215.5	69.0	69	8.70	88.7
5	0.03	173.2		-23.0		42.10	
6	0.03	158.0	165.6	-13.0	-18	12.60	27.3
7	0.05	129.0		-36.0		37.10	
8	0.05	216.0	172.5	-32.0	-34	37.20	37.2
9	0.10	288.0		-64.0		12.70	
10	0.10	302.0	295.0	-82.0	-73	6.62	9.6
11	0.15	504.0		-219.0		13.00	
12	0.15	647.0	575.5	-223.0		7.45	10.2
13	0.20	1,079.0		-18.0		10.00	
14	0.20	1,150.0	1,114.5	-40.0	-29	10.60	10.3
15	0.30	1,663.0		-420.0		10.10	
16	0.30	1,052.0	1,357.5	-466.0	-443	9.50	9.8
17	0.40	590.0		301.0		7.20	
18	0.40	559.0	559.0	511.0	406	7.20	7.2

Small amounts of  $\text{NaNO}_3$  had no apparent stimulating effect on nitrogen fixation. Up to a concentration of 0.15 per cent less nitrogen was found than in the checks. As the concentrations increased up to 0.3 per cent the amount of nitrogen fixed increased. At percentages higher than this there was a decided decrease. The results of  $\text{NaNO}_3$  on nitrification are not very consistent. From these results, it appears that nitrification is not only depressed but that there is actually a loss of nitrate. At the highest concentration, 0.4 per cent there was a gain in nitrates. This result corresponds with those of Dehrain (8) who found that nitrates may stop nitrification for a time, but later stimulate it.

Ammonification in this series was slightly inhibited by a concentration of 0.01 per cent of  $\text{NaNO}_3$ . The greatest increase was with a concentration of

0.05 per cent. At higher concentration there was a gradual falling off of ammonification and at 0.3 per cent it was inhibited. The toxic points of  $\text{NaNO}_3$  were 0.01 per cent for ammonification and 0.4 per cent for nitrogen fixation.

*Series 4. The effect of  $\text{Na}_2\text{SO}_4$  on nitrogen fixation, nitrification and ammonification*

In this series the concentration of the salt used is a great deal higher than in the case of any of the other series.

TABLE 6  
*Effect of  $\text{Na}_2\text{SO}_4$  on nitrogen, nitrification and ammonification*

NUMBER	$\text{Na}_2\text{SO}_4$	NITROGEN FIXATION		NITRIFICATION		AMMONIFICATION	
		Total nitrogen	Average	$\text{NO}_2$	Average	$\text{NH}_3$	Average
	<i>per cent</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>	<i>mgm. p. m.</i>
1	None	341		50.8		10.06	
2	None	341	341	89.2	70.0	9.90	9.9
3	0.1	1,172		115.0		2.05	
4	0.1	1,058	1115	119.1	117.0	7.10	4.5
5	0.2	1,150		107.0		14.20	
6	0.2	1,224	1187	74.0	90.5	10.20	12.2
7	0.4	3,230		85.9		17.40	
8	0.4	3,826	3528	86.0	86.0	12.20	14.8
9	0.6	1,228		71.0		10.70	
10	0.6	3,971	2599	74.1	72.0	7.40	9.0
11	1.0	2,080		69.0		10.90	
12	1.0	2,504	2292	73.0	71.0	13.10	12.0
13	2.0	2,519		68.8		12.20	
14	2.0	2,373	2446	66.0	67.4	12.10	12.1
15	3.0	3,240		60.0		11.30	
16	3.0	2,988	3114	57.0	58.5	11.90	16.6
17	4.0	1,526		38.0		14.70	
18	4.0	1,385	1455	27.6	32.8	19.70	17.2

These results showed rather conclusively that  $\text{Na}_2\text{SO}_4$  stimulated nitrogen fixation with all the different percentages used. The maximum occurred with a concentration of 0.4 per cent although the amount found at a concentration of 3 per cent was almost as large. Nitrification was increased by concentrations as high as 0.4 per cent. Above this concentration there was a gradual falling off. None of the concentrations used inhibited ammonification. The maximum amount took place at the highest concentration. Of all the salts used in this work  $\text{Na}_2\text{SO}_4$  proved to be the least toxic as measure by all three of the bacterial activities studied.

## CROP EXPERIMENTS

The crop experiments were carried on in two sets of series, with wheat and with field peas. The one set was carried on to determine the effect of single alkali salts on crop growth, the other when the alkali salts were used in combination as found under field conditions.

*Series 5. The effect of single alkali salts on the yield of wheat and of field peas.*

In this series wheat and peas were grown on soil that contained varying percentages of NaCl, Na<sub>2</sub>CO<sub>3</sub>, NaNO<sub>3</sub>, and Na<sub>2</sub>SO<sub>4</sub>, respectively. Where no yields are recorded, it was impossible to get the seeds to germinate.

TABLE 7

*Crop yields of wheat and of field peas on soil with different percentages of NaCl, Na<sub>2</sub>CO<sub>3</sub>, NaNO<sub>3</sub>, and Na<sub>2</sub>SO<sub>4</sub>*

NUMBER	SALT	YIELD OF WHEAT				YIELD OF PEAS			
		NaCl	Na <sub>2</sub> CO <sub>3</sub>	NaNO <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>	NaCl	Na <sub>2</sub> CO <sub>3</sub>	NaNO <sub>3</sub>	Na <sub>2</sub> SO <sub>4</sub>
	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1	None	116	116	116	116	221	221	221	221
2	0.1	98	89	118	112	160	172	276	255
3	0.2	80	130	108	71	226	207	275	178
4	0.4	22	106	75	183	000	202	229	229
5	0.6	00	137	49	98	000	000	000	164
6	0.8	00	175	00	116	000	000	000	230
7	1.0	00	95	00	62	000	000	000	236
8	2.0	00	00	00	50	000	000	000	000

According to these results, field peas are more sensitive to all of the alkali salts used than is wheat. NaCl in small amounts stimulated slightly the growth of peas, but at a concentration between 0.2 and 0.4 per cent, it was impossible even to get the seed to germinate. No stimulating effect was found on peas when Na<sub>2</sub>CO<sub>3</sub> was used, although crop growth took place at a higher concentration than in the case of the chloride. No growth took place at concentration above 0.4 per cent. As with the case of the chloride, NaNO<sub>3</sub> produced an increase in yield when low concentrations were used but at percentages above 0.4 per cent no growth occurred. At the different concentration at which growth did take place there was practically no difference in yield.

The least toxic of the four salts was Na<sub>2</sub>SO<sub>4</sub>. A stimulating effect was shown in all cases and growth took place at a concentration as high as 1 per cent. The increase in growth in the case of both the nitrate and the sulfate series undoubtedly is due to the nitrogen and sulfur contained in each respectively.

NaCl had no stimulating effect on the growth of wheat, and as the concentration increased the yield gradually decreased. The growth at 0.4 per cent was very poor and above this no germination took place. The falling off of



the yield in the case where  $\text{Na}_2\text{CO}_3$  was used was not very marked, and fairly good growth took place with a concentration of 1 per cent. At concentration above this the yield decreased very rapidly. No germination took place at 2 per cent.  $\text{NaNO}_3$  ranked next to the chloride in toxicity. Wheat seemed to be more sensitive to this salt than field peas, and although germinations took place at a concentration as high as 0.6 per cent the yields in most cases were below that of the checks. In the case of  $\text{Na}_2\text{SO}_4$ , the wheat germinated at a concentration of 2 per cent but no stimulating effect was noted as was the case with field peas.

*Series 6. The effect on crop yields of alkali salts in combination, NaCl predominating*

This series and all of the following series contained different percentages of salts as found by analyzing the water-soluble salts of an alkali soil. In this series NaCl was present in the largest amounts. The salts present and the yields of wheat and peas are shown in table 8.

TABLE 8

*Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL SALT	NaCl	$\text{CaSO}_4$	KCl	$\text{MgCl}_2$	$\text{CaCl}_2$	$\text{NaHCO}_3$	GERMINATION	YIELD OF WHEAT	YIELD OF PEAS
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	mgm.	mgm.
1	None	None	None	None	None	None	None	100	84.0	250
2	None	None	None	None	None	None	None	100	115.0	206
3	0.1	0.08	0.006	0.004	0.007	0.0002	0.0003	100	163.6	195
4	0.2	0.16	0.012	0.008	0.014	0.0004	0.0006	75	145.6	202
5	0.4	0.32	0.024	0.016	0.028	0.0008	0.0012	25	53.0	86
6	0.6	0.48	0.036	0.024	0.042	0.0012	0.0018		00.0	00
7	0.8	0.64	0.048	0.032	0.056	0.0016	0.0024		00.0	00
8	1.0	0.80	0.060	0.040	0.070	0.0020	0.0030		00.0	00
9	2.0	1.60	0.120	0.080	0.140	0.0040	0.0060		00.0	00

In this series the field peas made a poor growth at a percentage higher than that where NaCl was used alone. This may be due to the fact that small amount of  $\text{CaSO}_4$  was present. Wheat on the other hand stopped growth at a lower concentration than in the case where the single salt was used. Both this series and Series 1 and 5 indicate that NaCl is the most toxic of all the salts tested.

*Series 7. The effect of crop yields on alkali salts in combination,  $\text{Na}_2\text{CO}_3$  predominating*

In this series  $\text{Na}_2\text{CO}_3$  made up about 75 per cent of the salts and there was about 12 per cent of  $\text{NaNO}_3$  with small amounts of the chloride and sulfate present.

TABLE 9

*Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL SALT	Na <sub>2</sub> CO <sub>3</sub>	NaNO <sub>3</sub>	Na <sub>2</sub> PO <sub>4</sub>	Na <sub>2</sub> SO <sub>4</sub>	NaCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>
1	None	None	None	None	None	None	100	84	250
2	None	None	None	None	None	None	100	115	206
3	0.1	0.075	0.013	0.005	0.00467	0.0014	100	148	175
4	0.2	0.150	0.026	0.010	0.00934	0.0029	100	60	162
5	0.4	0.300	0.052	0.02	0.00868	0.0058	50	75	152
6	0.6	0.450	0.078	0.03	0.02802	0.0087	50	132	140
7	0.8	0.600	0.104	0.04	0.03736	0.0116		122	00
8	1.0	0.750	0.130	0.05	0.04670	0.0146		00	00
9	2.0	1.500	0.260	0.10	0.09340	0.0292		00	00

In this series the peas again showed a greater resistance to the carbonate than when the carbonate was used alone. This perhaps is due to the small quantities of NaNO<sub>3</sub>. Wheat stopped growth at a concentration lower than when NaCO<sub>3</sub> was used alone. Both of these results are in accord with the results in Series 2 and 5. NaNO<sub>3</sub> is more favorable to the legume than to wheat.

*Series 8. The effect on crop yields of alkali salts in combination, Na<sub>2</sub>SO<sub>4</sub> predominating*

In this series there were two alkali salts present, Na<sub>2</sub>SO<sub>4</sub> and NaCl. There was 94 per cent of the sulfate and 6 per cent of the chloride.

TABLE 10

*Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL	Na <sub>2</sub> SO <sub>4</sub>	NaCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>
1	None	None	None	100	84.0	250
2	None	None	None	100	115.0	206
3	0.10	0.093	0.006	100	229.6	178
4	0.20	0.186	0.013	100	162.4	238
5	0.40	0.373	0.026	100	150.0	178
6	0.60	0.560	0.039	100	143.0	224
7	0.80	0.747	0.052	100	115.0	117
8	1.00	0.934	0.066	100	90.0	161
9	2.00	1.868	0.132		00.0	00

Crop growth took place in a concentration as high as 0.8 per cent of the total salt without any apparent decrease in yield. At 1 per cent there was a slight decrease in the case of both the wheat and the field peas, and at a

higher concentration no growth took place. The toxic point in this series is lower than where  $\text{Na}_2\text{SO}_4$  was used alone. This undoubtedly is due to the  $\text{NaCl}$  present.

*Series 9. The effect on crop yields of alkali salts in combination  $\text{Na}_2\text{SO}_4$  predominating*

In this series as in Series 8  $\text{Na}_2\text{SO}_4$  predominated, but it made up only about 58 per cent of the total salt. The other 42 per cent was made up of the carbonate and chloride of sodium.

TABLE 11

*Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL SALT	$\text{Na}_2\text{SO}_4$	$\text{Na}_2\text{CO}_3$	$\text{NaCl}$	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>
1	None	None	None	None	100	84.0	250
2	None	None	None	None	100	115.0	206
3	0.1	0.058	0.022	0.018	100	132.4	221
4	0.2	0.117	0.045	0.037	50	134.2	90
5	0.4	0.234	0.091	0.074	100	103.5	247
6	0.6	0.351	0.137	0.111	75	23.0	139
7	0.8	0.468	0.183	0.148	25	84.0	48
8	1.0	0.585	0.229	0.186	25	49.0	50
9	2.0	1.170	0.488	0.372		00.0	00

These results show that as in the case of the previous series both peas and wheat germinated at a concentration as high as 1 per cent. The germination and yield, though at the higher concentrations, were considerably less than in series 8. This injurious effect is undoubtedly due to the presence of both the chloride and carbonate.

*Series 10. The effect on crop yields of alkali salts in combination  $\text{NaHCO}_3$  predominating*

The salts used in this series were chiefly the carbonates and bi-carbonates of sodium, potassium and calcium with a small amount of  $\text{CaSO}_4$ . The bi-carbonate of sodium predominated in this combination.

Peas would not germinate at a concentration of 0.8 per cent and wheat made very little growth at this same concentration. These percentages are lower than the toxic points found when  $\text{Na}_2\text{CO}_3$  was used alone. These results would indicate that the bi-carbonate is more toxic than the carbonate.

TABLE 12

*Yield on wheat and field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL SALT	NaHCO <sub>3</sub>	KHCO <sub>3</sub>	CaCO <sub>3</sub>	CaSO <sub>4</sub>	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	per cent	per cent	per cent	per cent	per cent	mgm.	mgm.
1	None	None	None	None	None	100	84.0	250
2	None	None	None	None	None	100	115.0	206
3	0.1	0.090	0.002	0.003	0.004	50	85.0	96
4	0.2	0.181	0.004	0.006	0.008	75	124.0	144
5	0.4	0.362	0.007	0.012	0.016	100	136.0	206
6	0.6	0.544	0.012	0.018	0.024	100	119.0	196
7	0.8	0.725	0.016	0.024	0.032		81.2	00
8	1.0	0.907	0.021	0.030	0.040		00.0	00
9	2.0	1.814	0.042	0.060	0.080		00.0	00

*Series 11. The effect on crop yields of alkali salts in combination NaNO<sub>3</sub> predominating*

In this series the salts used were the nitrate, carbonate and chloride of sodium. Sodium nitrate made up 87 per cent of the total.

TABLE 13

*Yield of wheat and of field peas on soil having alkali salts in combination as found under field conditions*

NUMBER	TOTAL SALT	NaNO <sub>3</sub>	Na <sub>2</sub> CO <sub>3</sub>	NaCl	KCl	GERMINATION-PEAS	YIELD OF WHEAT	YIELD OF PEAS
	per cent	per cent	per cent	per cent	per cent	per cent	mgm.	mgm.
1	None	None	None	None	None	100	84	250
2	None	None	None	None	None	100	115	206
3	0.1	0.087	0.004	0.002	0.006	100	89	229
4	0.2	0.175	0.008	0.005	0.012	100	110	235
5	0.4	0.348	0.016	0.010	0.024	75	103	133
6	0.6	0.522	0.024	0.015	0.036		00	00
7	0.8	0.696	0.032	0.020	0.048		00	00
8	1.0	0.870	0.040	0.025	0.060		00	00
9	2.0	1.740	0.000	0.051	0.020		00	00

This combination was the most toxic one used and is in accordance with the results found where the individual salts were used. Neither germination nor crop growth took place at a concentration above 0.4 per cent.

## DISCUSSION

The results of this investigation on the effect of crop growth and bacterial activities of alkali salts, although carried on under laboratory conditions, can be used to advantage for planning field work on this problem.

The bacteriological tests checked fairly well with the crop growth and in most cases accorded with the results obtained by J. G. Lipman, P. E. Brown, C. B. Lipman and W. P. Kelley, except in the points of toxicity for an individual salt.

The wide variations in the point of toxicity were almost lacking, although the author did not use any of the compounds like dextrose, blood meal or cotton seed meal,  $(\text{NH}_4)_2\text{SO}_4$  for nitrogen fixation, ammonification and nitrification. The use of such material was intentionally avoided so as to compare as closely as practicable, the activities of organisms in the presence of these injurious salts under the actual crop-growing conditions.

The little variation in the degree of toxicity may be due to such causes as: (a) The variation in manipulation in the experimental technique (b) The difference in soil composition (c) and above all the different bacterial flora with varying vigor present in different soils, under varying local climatic conditions.

#### SUMMARY

1. In order of toxicity the salts rank as follows:  $\text{NaCl}$ ,  $\text{NaNO}_3$ ,  $\text{Na}_2\text{CO}_3$  and  $\text{Na}_2\text{SO}_4$ . The per cent of the anion and not the cation is the determining factor.

2. Small amounts of each of the different salts used stimulated both crop growth and bacterial activities. This amount varied with the crop grown, and the concentrations at which stimulation took place bore the same relationship to each other as did their toxicity points.

3.  $\text{NaCl}$  became toxic to both ammonification and nitrogen fixation at 0.01 per cent. The toxic point for wheat was 0.4 per cent and for field peas 0.2 per cent.

4.  $\text{NaNO}_3$  became toxic to ammonification at a concentration of 0.01 per cent and to nitrogen fixation above 0.4 per cent. Small concentrations of  $\text{NaNO}_3$  proved toxic to nitrification but at a concentration of 0.4 per cent a marked stimulation took place. The toxic points for wheat and for peas were 0.8 and 0.6 per cent, respectively.

5.  $\text{Na}_2\text{CO}_3$  was toxic to ammonification at a concentration of 0.02 per cent, to nitrification at 0.8 per cent and to nitrogen fixation at 0.01 per cent. For wheat the toxic point was 1 per cent and for peas 0.6 per cent.

6.  $\text{Na}_2\text{SO}_4$  proved to be the least toxic of all the salts. Neither ammonification or nitrogen fixation was inhibited to any extent at concentrations up to 2 per cent and peas up to between 1 and 2 per cent.

7. The toxicity point as found when salts were used in combination, as under field conditions, checked very closely with the points found when the individual salts were present. The toxic point of the combined salts depended upon the percentages of the chlorides, nitrates, carbonates and sulfates present, and the combination in which they exist.  $\text{CaSO}_4$ , when present, lowered the toxic point of the chloride, carbonate and nitrate of sodium.

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## ABNORMAL STEM GROWTH OF SOYBEANS IN SAND CULTURES WITH SHIVE'S THREE-SALT SOLUTION

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In a recent paper (1) Shive has shown that several monobasic phosphate salts, when added to soil or to solution cultures, are toxic to soybean plants growing in the media. This toxicity not only retarded the growth of the plants but also produced certain specific injuries to the plant tissue. These tests involved three sets of experiments: (a) plants grown in soil cultures to which the salts were added singly in solutions; (b) plants grown in soil cultures to which the phosphates were applied in connection with a complete fertilizer treatment; and (c) plants grown in solution cultures in which the mixed solutions employed in the soil series were used without alteration.

Each of the phosphates used singly in his soil cultures caused specific injury to the soybean plants when the initial concentration of the solution was above one atmosphere, the mono-potassium phosphate being least injurious and the mono-calcium phosphate producing the greatest injury. The plants from a number of soil cultures in which the phosphates were used in connection with a complete fertilizer also suffered pronounced injury, while the plants grown in solution cultures sustained more pronounced injuries than those grown in the corresponding soil cultures.

The nature of the injury sustained by the soybean plants grown by Shive in the different culture media, was identical and appeared to be directly related to some property common to all of the solutions employed. The injury was first marked by a retardation in growth, after which, evidence of specific injury appeared in the form of dark brown discolorations around the margins of the cotyledons. In cases of severe injury the discoloration spread rapidly over the entire surface of the cotyledons and soon appeared on the foliage leaves. Leaf injury appeared first near the margin in the form of small yellowish, round spots which rapidly turned brown. In severe cases the spots gradually increased in size until they covered the entire leaf surface, causing death and the falling of the organ.

In his soil cultures with complete fertilizer rations evidence of disturbed growth did not appear until the third week of the growth period. The group of cultures to which Shive's original three-salt solution alone was added, suffered no specific injury.



The growth period for these cultures extended over a period of 30 days.

During the past two years, the writers have had occasion to study the behavior of the soybean plant growing in sand cultures and supplied with Shive's 3-salt solution having a total initial osmotic concentration of 1.75 atmospheres. Using this 3-salt solution it was desired to determine the proportions of mono-potassium phosphate, calcium nitrate and magnesium sulfate that would give the best growth of the soybean plant at different stages of its development.

Since the dry weight was to be used as a measure of the growth rate during the different periods, it was of course impossible to carry through to maturity the same individuals. According to the plan adopted, the first series of cultures was grown for a period of 30 days, when it was harvested and the dry weights determined. A second series of cultures was then started from the same lot of seed as that used for the first series. During the first 30 days these plants of the second series were given a nutrient solution containing the same proportion of the three salts as had been found to give the best growth rate during the first growth period. From the close of the first 30-day period to the end of 60 days the individual cultures of the second series were supplied with the same different proportions of the three salts as were employed in the first series. At the end of 60 days the second series was harvested and from the dry weights the best proportion of salts for the second period was obtained. In a similar manner the plants for the third series were to be grown from the same lot of seed and supplied with the best proportions of salts during the first and second periods, after which they were to be given the differential feeding and harvested at maturity.

Ten days after transplanting the first series, the plants in the cultures receiving solutions high in mono-potassium phosphate began to show signs of specific injury. When viewed from above the first leaves (the cotyledons having been removed) were marked by dark brown spots. When viewed from below these leaves showed a distinct enlargement and reddening of the midribs and veins (plate 1). The leaves that developed later were of a pale green color and exhibited the characteristic reddening of the veins on the under side.

Of this series, culture  $R_2C_6$  gave the largest dry weight yield. This culture was supplied with a solution having two-tenths of its total concentration derived from mono-potassium phosphate, six-tenths from calcium nitrate and two-tenths from magnesium sulfate. After the first series was harvested the second lot of plants was started and all of the cultures were supplied alike with the above mentioned proportions of the three salts used during the first 30-day period, after which the differential feeding was inaugurated. An inspection of the plants at this time revealed no evidence of specific injury although a few plants appeared to have been injured at the point where the cotyledons were removed.

At the middle of the second growth period, when the plants were approximately 45 days old, the specific injuries which had characterized certain

cultures in the first growth period made their appearance. In this instance, however, the injury was not confined to the plants growing in the cultures receiving solutions high in mono-potassium phosphate. As growth proceeded the injury to the leaves became more pronounced and was accompanied by a thickening of the stems to approximately twice the normal size and a gradual cessation of the growth in height. Plate 2 shows the characteristic appearance of the plants at the end of the 60-day growth period. An examination of cross-sections taken through the affected regions, revealed the fact that the increased diameter of the stem was not the result of a thickening of a particular tissue, and showed that growth in thickness had increased in all of the tissue of the swollen stems except the extreme outer layers. The thickening was less pronounced, however, in the pith than in the vascular ring. Starch was abundant in the swollen tissue. The cultures in which the most pronounced injury occurred were as follows:

$R_1C_7$  with one-tenth of its total concentration derived from mono-potassium phosphate, seven-tenths from calcium nitrate and two-tenths from magnesium sulfate;  $R_2C_8$  with two-tenths from mono-potassium phosphate, three-tenths from calcium nitrate and five-tenths from magnesium sulfate;  $R_3C_5$  with three-tenths from mono-potassium phosphate, five-tenths from calcium nitrate and two-tenths from magnesium sulfate;  $R_3C_6$  with three-tenths from mono-potassium phosphate, six-tenths from calcium nitrate and one-tenth from magnesium sulfate.

Since this injury was present in cultures growing in solutions of such widely varying salt proportions, it would appear that instead of being correlated with a high concentration of a particular salt, the abnormal growth in the soybean plants must have been the result of some property which was common to all of the Shive 3-salt solutions.

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PLATE 1

CHARACTERISTIC ENLARGEMENT OF MIDRIBS AND VEINS OF SOYBEAN PLANTS GROWN IN  
SAND CULTURES WITH SHIVE'S THREE-SALT SOLUTION. *A* AND *C*, UNDER  
SIDE; *B*, UPPER SURFACE; *D*, NORMAL LEAF

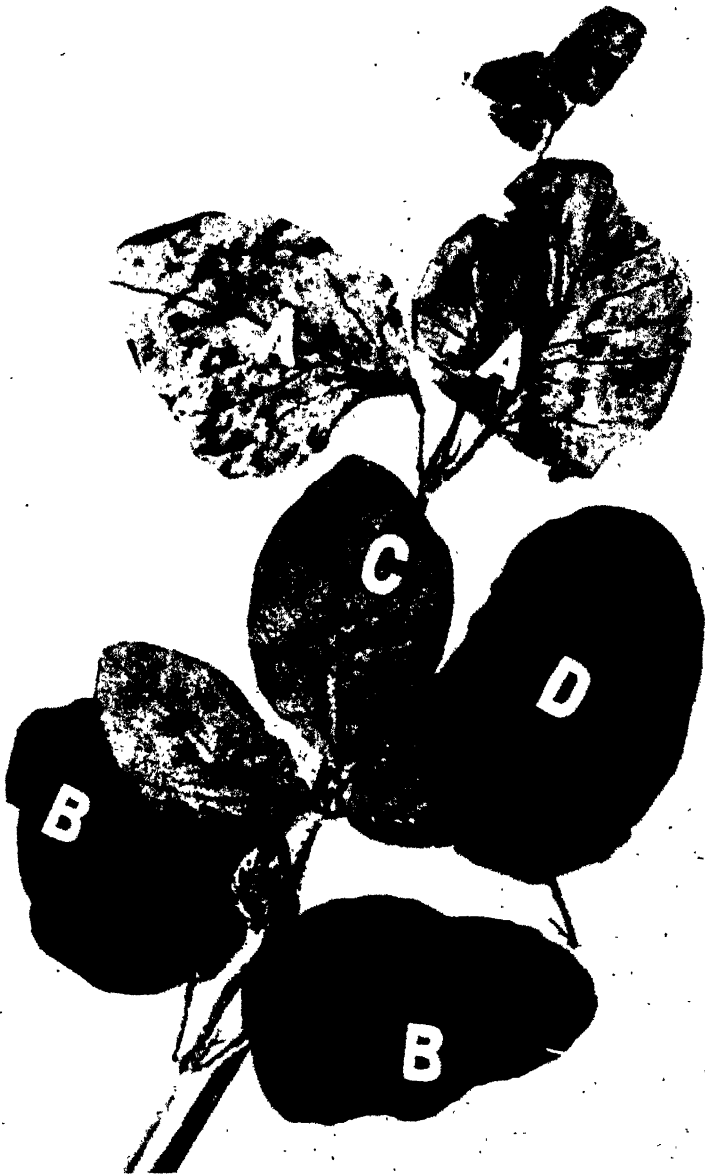


PLATE 2

ABNORMAL STEM DEVELOPMENT OF SOYBEAN PLANTS GROWN IN SAND CULTURES WITH  
SHIVE'S THREE-SALT SOLUTION





# THE DETERMINATION OF TOTAL NITROGEN IN SOILS CONTAINING RATHER LARGE AMOUNTS OF NITRATES

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In the course of some experimental work on soils kept in the greenhouse, it was found that the nitrate content was rather high, varying from 22 to 153 parts of nitrogen per million. The total nitrogen in the soils varied from 0.125 per cent to 0.156 per cent. The nitrate nitrogen, therefore, amounted to 1.6 per cent of the total nitrogen in the lower case and to 10 per cent in the higher, an amount which should not be overlooked in determining the total nitrogen.

These results confirmed the conclusions of Lyon and Bizzell<sup>2</sup> who found from their own observations and the investigations of others that the nitrate nitrogen even in ordinary cropped and uncropped soils ran all the way from 1 to 50 parts of nitrogen per million, and should certainly be included as a part of the total nitrogen.

There are various methods for determining the total nitrogen in soils, both with and without the inclusion of the nitrates, and several of them were studied to determine their relative accuracy.

The soil taken for analysis contained 145 parts of nitrate nitrogen per million, as determined by the aluminum reduction method, which was the method employed for all the nitrate determinations.

In the first test, four methods for total nitrogen were used, three of which were for total nitrogen including nitrates, and the other simply for total nitrogen. The purpose of the test was to determine whether this later method recovered any or all of the nitrates present. These four methods were the "Ulsch" method, the "Salicylic" method, the "Aluminum" method and the "Hibbard" method. As each of these methods was somewhat modified, a brief statement of procedure will be given here.

*Ulsch method.* Add 100 cc. ammonia free water to soil, 40 cc. dilute  $H_2SO_4$  (1-2 of  $H_2O$ ), add 1 gm. iron slowly, boil 5 minutes, add 60 cc. concentrated  $H_2SO_4$ , evaporate and drive off fumes; add  $K_2SO_4$  and  $CuSO_4$  mixture and digest. Neutralize and determine ammonia.

*Salicylic method.* 120 cc. concentrated  $H_2SO_4$  containing 8 gm. salicylic acid. Add slowly 4 gm. zinc dust, drive off fumes, then add  $K_2SO_4$  and  $CuSO_4$  mixture and digest.



*Hibbard method.* Drive off fumes with 75 cc. concentrated  $\text{H}_2\text{SO}_4$ , add  $\text{K}_2\text{SO}_4$ ,  $\text{CuSO}_4$ , and  $\text{FeSO}_4$  mixture and digest.

*Aluminum method.* Add 150 cc.  $\text{H}_2\text{O}$ , 15 cc. of 10 per cent  $\text{NaOH}$  and 1 gm. of aluminum to soil. Let digest 24 hours, connecting up Kjeldahl with an Erlenmeyer flask which contains 50 cc. of 10 per cent  $\text{H}_2\text{SO}_4$  to catch any  $\text{NH}_3$  fumes driven off. Pour the 10 per cent  $\text{H}_2\text{SO}_4$  into the Kjeldahl, add 80 cc. concentrated  $\text{H}_2\text{SO}_4$ , evaporate and drive off fumes, then digest, adding Hibbard mixture.

Twenty-five grams of soil were used in all determinations and each soil was run in quadruplicate.

The widest variation of any one from the average of all the determinations was not over 0.2 of a cc. of  $\text{N}/10$  acid.

	AVERAGE NITROGEN	NITROGEN
	gm.	per cent
The Ulsch method.....	0.038353	0.153
The Salicylic method.....	0.039387	0.158
The Aluminum method.....	0.039071	0.156
The Hibbard method.....	0.038784	0.155

This showed at once, that in this particular soil, which contained 10 per cent of its total nitrogen in the form of nitrates, either a reduction of nitrates occurred in the Hibbard method which was not supposed to determine nitrate nitrogen as a part of the total nitrogen, or else none of the methods supposed to include nitrates actually did so.

In the next test a soil which contained no nitrates, as determined by the aluminum reduction method, was employed and total nitrogen determinations were run both with the Hibbard method and the mercury method (the usual method for total nitrogen which is not supposed to include nitrates); with and without the addition of known amounts of nitrates.

(25 gm. soil)	AVERAGE NITROGEN	NITRATE NITROGEN RECOVERED
	gm.	gm.
Hibbard method soil.....	0.042906	
Hibbard method soil + 0.00375 gm. nitrogen as $\text{KNO}_3$ .....	0.046584	0.00368
Mercury method soil.....	0.041400	
Mercury method soil + 0.00375 gm. nitrogen as $\text{KNO}_3$ .....	0.045148	0.00375

These results show conclusively that all the nitrates added to this soil were recovered by the Hibbard and mercury methods. It is evident that in this soil a reduction of nitrates was brought about without the addition of any special reducing agents.

It is possible that some compound in the organic matter of the soil has the same property of reacting with the nitrates as salicylic acid (ortho-hydroxy-

benzoic acid). After such a nitration has been effected, sulphur dioxide, generated by the action of the organic matter on the  $\text{H}_2\text{SO}_4$ , may become the agent reducing the nitrogen compound. In the soil there undoubtedly exist ring compounds such as tyrosine, which have a hydroxy group in the phenyl ring just like salicylic acid, and hence would react similarly. There are also, in all probability, other benzol compounds in the soil which would act in much the same way.

Another test was run to determine whether in soils fairly low in organic matter the nitrates would be recovered by the unmodified methods.

Total nitrogen determinations were made in triplicate on three soils of varying carbon content with and without the addition of 3 mgm. of nitrate nitrogen per sample—an equivalent of 150 parts per million. These determinations were all made by the Hibbard method.

(20 GM. SOIL)	CARBON	NITRATE ADDED	AVERAGE NITROGEN	NITRATE NITROGEN RECOVERED
	<i>per cent</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Soil 50181.....	0.836		0.016252	
Soil 50181.....	0.836	0.0030	0.019417	0.00316
Soil 50191.....	0.412		0.009331	
Soil 50191.....	0.412	0.0030	0.012133	0.00280
Soil 50281.....	0.188		0.006487	
Soil 50281.....	0.188	0.0030	0.008126	0.00164

These results show that in soils very low in organic carbon the nitrates are not reduced by this method, and a modified method to include nitrates must be used. However, except in the case of extremely sandy soils and subsoils, most soils do not run below 0.4 per cent carbon, and on an average they run much higher (0.8 to 3 per cent), so that ordinarily the common methods for total nitrogen will recover the nitrate nitrogen.

The following conclusions may be drawn from this paper:

1. Many soils have a high nitrate content (up to 10 per cent of the total nitrogen) and the recovery of this nitrate is necessary in total nitrogen determinations.

2. If the organic matter of the soil is within the usual average (0.8 to 3.0 per cent), it is not necessary to use the modified methods for total nitrogen, the common unmodified methods (Hibbard or mercury) giving quite as accurate results.

3. Methods for total nitrogen, modified to include the nitrogen of nitrates must be employed, however, if the soils are lower than 0.5 to 0.6 per cent of organic carbon.

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